SynthAssist® Software

Version 3.1

User Guide

Applied Biosystems

For Research Use Only. Not for use in diagnostic procedures.

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Preface

About This Guide

Purpose of This Guide

The Applied Biosystems *SynthAssist® Software Version 3.1 User Guide* is for experienced SynthAssist software users migrating to the personal computer (PC) version or upgrading their SynthAssist software and for novice users. This document begins with a description of the SynthAssist software, the hardware and software requirements for operating the software, instructions for installing the software and for connecting the synthesizer to a PC. The remainder of the guide provides instructions on how to set up peptide synthesis runs, program the synthesizer, and collect data.

Audience

This guide is intended for experienced SynthAssist software users migrating to the personal computer (PC) version of the SynthAssist system or upgrading their SynthAssist software and for novice SynthAssist software users.

Assumptions

This guide uses conventions and terminology that assumes a working knowledge of the Microsoft Windows® operating system.

Text Conventions

This guide uses the following conventions:

- **Bold** indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow bracket (>) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File > Open > Spot Set**.

  Right-click the sample row, then select **View Filter > View All Runs**.

User Attention Words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Examples of the user attention words appear below:

Note: If you select the C- or N-terminus of the sequence in the Sequence screen, the palette changes to a selection of C- or N-termini.

IMPORTANT! You can copy the amino acids in a sequence but not the termini.

How to Obtain More Information

Related Documentation

SynthAssist® 3.1 Online Help – Describes the SynthAssist version 3.1 software and provides procedures for common tasks.

Note: For additional documentation, see "How to Obtain Services and Support" on page ix.

Send Us Your Comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

How to Obtain Services and Support

To contact Applied Biosystems Technical Support from North America by telephone, call **1.800.899.5858**.

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Services and Support**.

At the Services and Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Services and Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

Quick Start Checklist 1

Use this checklist to:

- Install your SynthAssist® Software Version 3.1
- Set up your chemistry
- Set up a peptide run

See the referenced sections for detailed procedures.

☐ **Connect the communication cable.**

See “Connecting the ABI 433A Peptide Synthesizer to the Computer” on page 2-5.

☐ **Install SynthAssist 3.1 software.**

See “Installing SynthAssist Software Version 3.1” on page 2-6.

☐ **Start SynthAssist 3.1 software.**

See “Starting SynthAssist 3.1” on page 2-14.

☐ **Run a flow test.**

See “Running Flow Tests” on page 4-7.

☐ **Specify the sequence of amino acids.**

See “Specifying the Sequence of Amino Acids” on page 5-2.

☐ **Set up a peptide run.**

See “Setting Up a Peptide Run” on page 6-2.

☐ **Download chemistry and run a peptide synthesis.**

See “Sending a Peptide Synthesis” on page 6-10.

Introduction 2

This chapter covers:

Description of SynthAssist Software Version 3.1

SynthAssist® Software Version 3.1 is a peptide synthesis software system that runs on a PC and communicates with the ABI 433A Peptide Synthesizer. With SynthAssist software you can set up peptide synthesis runs, program the synthesizer, and collect data. The software is structured into the following subprograms:

Dictionary – A database of compounds used in peptide synthesis, including amino acids, other derivatives, resins, specified N- and C-terminals, and protecting groups.

Sequence – Allows you to type in or import peptide sequences, modify them, and perform simple calculations (for example, molecular weight and composition).

Chemistry – Allows you to set up the bottle configuration, user functions, cycles, modules, and runs.

Run – Merges sequence and chemistry information to create instructions for making a specified peptide on a specific instrument. It tracks the status of the peptide synthesizer and its current run.

Communications – Allows you to transfer modules, functions, runs, and status information between the PC and the synthesizer.

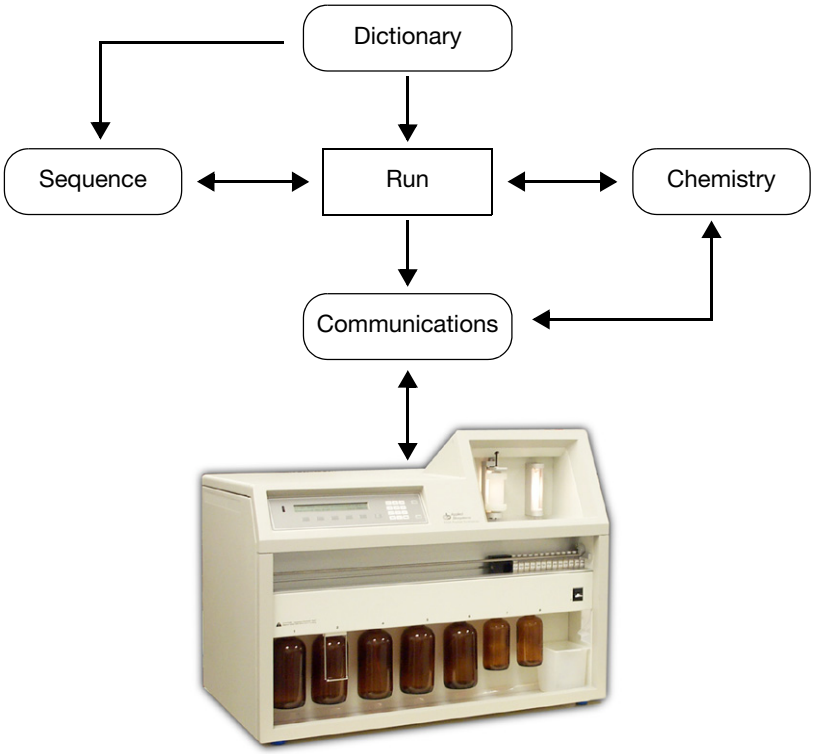


Figure 2-1 Software Subprogram Relationships

Hardware and Software Requirements

Computer SynthAssist Software 3.1 has been tested on Intel® Pentium III (1 GHz) or Intel® Pentium IV (2.4 or 2.8 GHz) processors. No attempt has been made to evaluate other hardware.

RAM At least 256MB of RAM.

Hard Disk Capacity At least 1 GB of free hard disk space.

Monitor A 17-inch flat-panel color monitor with the following display properties:

- Resolution – 1024 × 768
- Fonts – Small Fonts
- Color – True Color (16 bit)

System Software SynthAssist 3.1 Software can be installed on a PC running either Microsoft Windows® XP Professional (SP1 or higher) or Microsoft Windows® 2000 Professional (SP3 or higher).

Peptide Synthesizer The ABI 433A Peptide Synthesizer.

Communications Cable DB25 M to DB9 F W/FERRITE 10FT (Part Number 4342732)

Software The *SynthAssist 3.1 Software CD* contains the SynthAssist software installation program.

Connecting the ABI 433A Peptide Synthesizer to the Computer

Connect the communications cable from Port A on the ABI 433A Peptide Synthesizer to COM 1 on the computer as shown below in Figure 2-2.

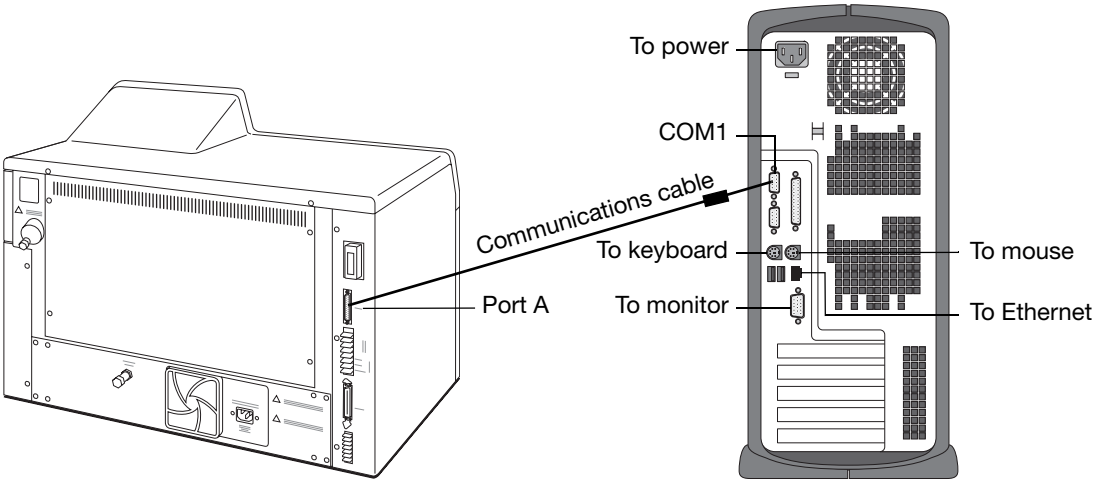


Figure 2-2 Installed Configuration

IMPORTANT! Keep the communications cable away from power cords.

Installing SynthAssist Software Version 3.1

Note: If any problems arise during the installation, cancel the process and remove the Installation CD. Delete the SynthAssist Software 3.1 application files via **Start>Settings>Control Panel>Add/Remove Programs**. Re-insert the Installation CD to start the install process again.

Note: If SynthAssist Software 3.0 is installed but is not found at C:\Program Files\Applied Biosystems\SynthAssist 3.0, then you must delete the SynthAssist Software 3.0 application files via **Start>Settings>Control Panel>Add/Remove Programs**. Note that the user-created files will remain. Re-insert the Installation CD to start the install process again.

IMPORTANT! Under no circumstances should the Version 3.0 and Version 3.1 applications be present simultaneously on the hard drive.

To install SynthAssist Software Version 3.1:

1. Insert the *SynthAssist 3.1 Software CD* into the CD drive of your computer.

   If autorun is enabled, the Preparing to Install screen is displayed. If the screen is not displayed, click **setup.exe** in the CD root directory to begin installation or re-start the computer.

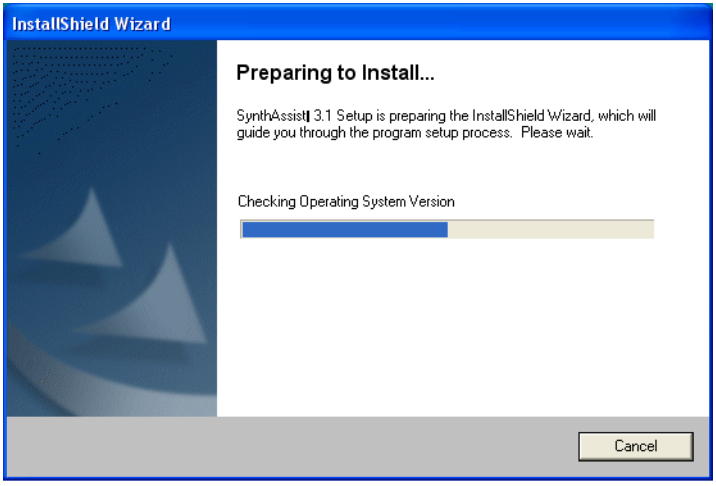


Figure 2-3 The Preparing to Install Screen

2. At the Welcome screen (Figure 2-4), click **Next** to open the Customer Information screen (Figure 2-5).

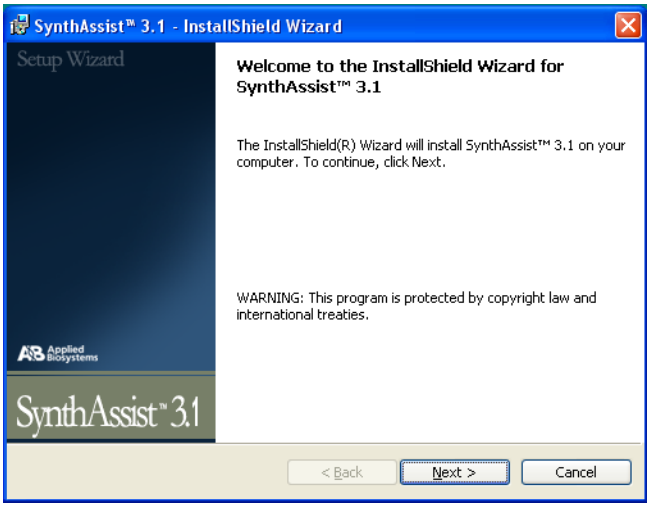


Figure 2-4 The Welcome Screen

3. Enter your User Name and Organization, then click **Next**.

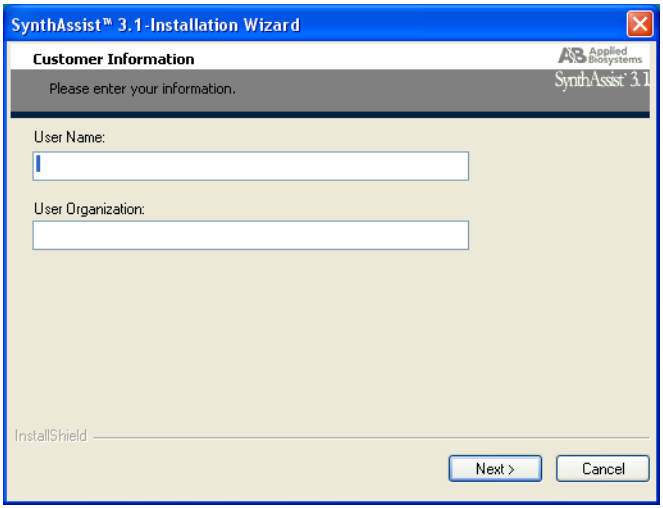


Figure 2-5 The Customer Information Screen

4. Click to accept the license agreement.
5. In the Destination Folder screen (Figure 2-6), click **Next** to accept the default location.

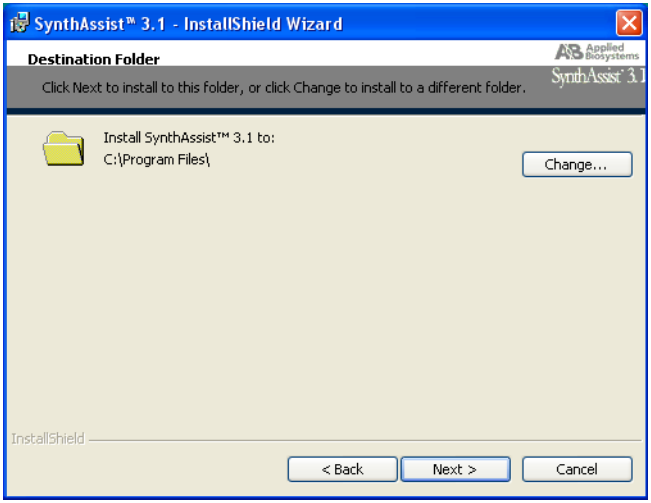


Figure 2-6 The Destination Folder Screen

Note: Always accept the default location, C:\Program Files\.

6. Click **Install** on the Ready to Install the Program screen (Figure 2-7) to begin the installation of SynthAssist Software 3.1.

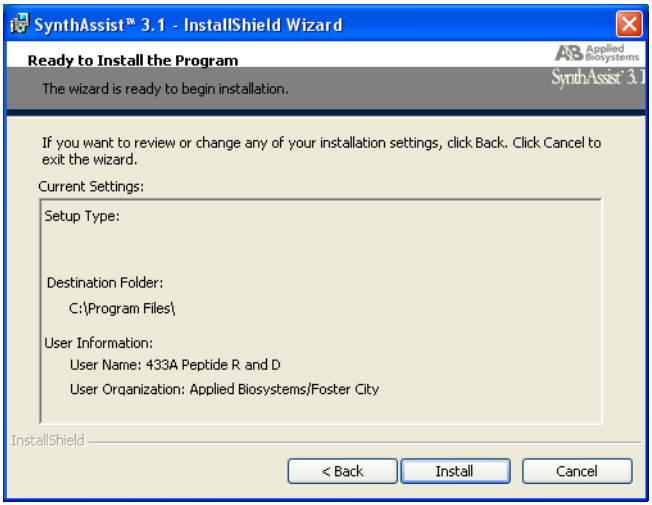


Figure 2-7 The Ready to Install the Program Screen

The Installing SynthAssist Software 3.1 screen (Figure 2-8) tracks the progress of the installation.

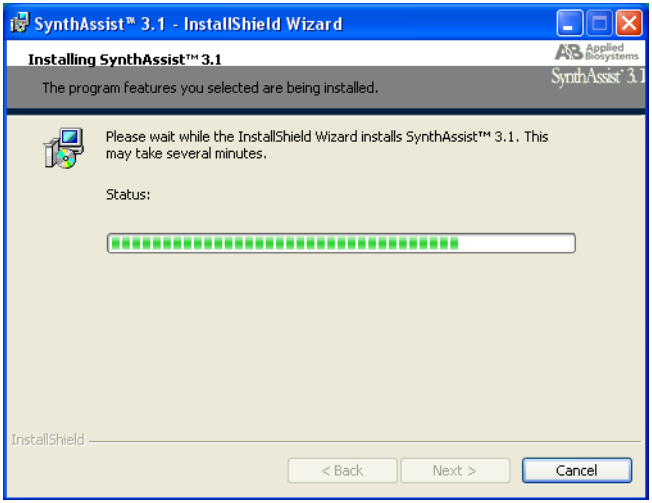


Figure 2-8 The Installing SynthAssist Software 3.1 Screen

IMPORTANT! If you are installing SynthAssist Software for the first time, skip directly to step 9. If you are upgrading to SynthAssist Software Version 3.1 from SynthAssist Software Version 3.0, continue to step 7.

7. The Detecting Previous Installation screen appears over the Installing SynthAssist Software 3.1 screen. Click **Ok** to continue.

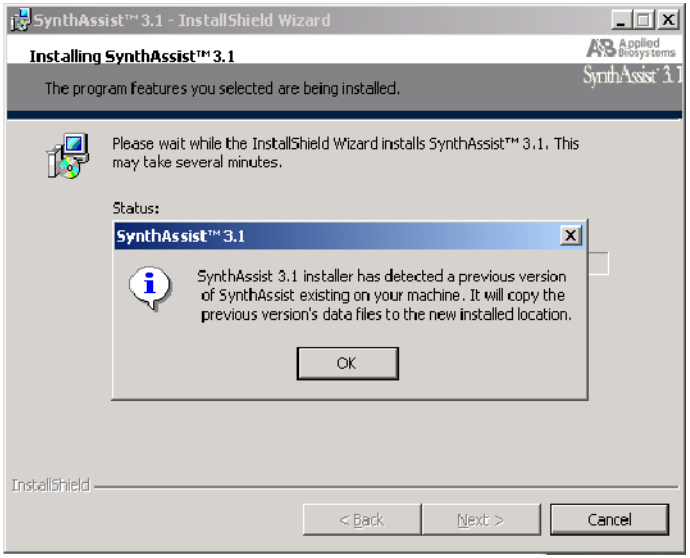


Figure 2-9 The Detecting Previous Installation Screen

The installation now removes SynthAssist Software 3.0, leaving the Chemisty, Run and Sequence folders. It moves each data file to the appropriate folder in SynthAssist 3.1.

8. Click **Ok** on the Files Copied Notification screen.

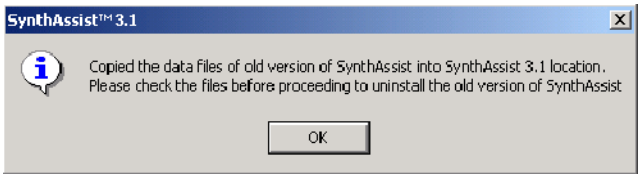


Figure 2-10 The Files Copied Notification Screen

9. Click **Finish** to complete the installation process.

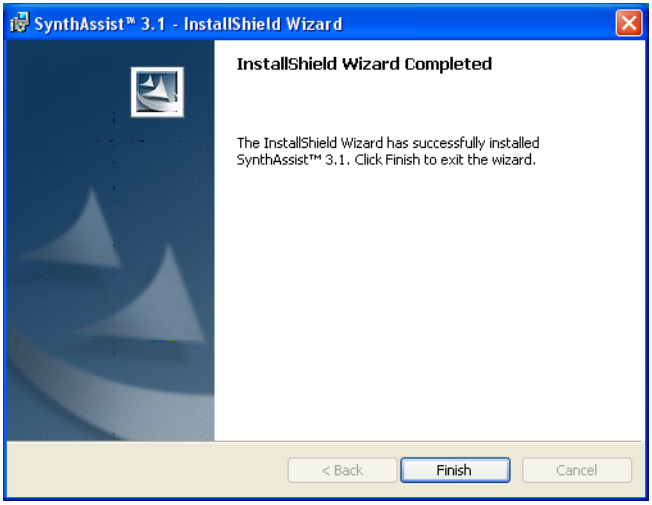


Figure 2-11 The Wizard Completed Screen

Shortcuts

A successful installation creates two shortcuts to SynthAssist Software 3.1:

- A desktop icon

- A Start menu path:
  Start > Programs > Applied Biosystems > **SynthAssist 3.1**

Folder Structure

The folder structure of SynthAssist Software 3.1 is displayed in Figure 2-12. (**C:\Program Files\Applied Biosystems**)

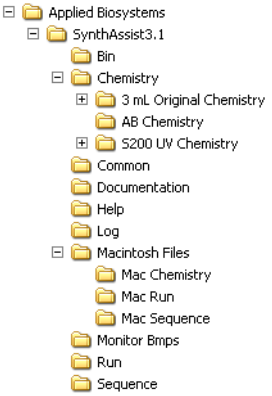


Figure 2-12 SynthAssist Software 3.1 Folders

Folder Contents

Bin

Contains the application and the dynamic link library files necessary to run SynthAssist Software 3.1. These are the only files that should be in the Bin folder. Never store other files in Bin.

Chemistry

Contains the standard AB conductivity chemistry files, any new chemistry files created with SynthAssist Software 3.1 or copied from Version 3.0, and any converted Macintosh® chemistry files. This folder contains two additional sub-directories:

- the 3 mL Original Chemistry (conductivity, PNA)
- the Series 200 UV Detector Chemistries

Common

Contains the SynthAssist Software Version 3.1 Dictionary, Lab, and Log files. Never store other files in Common.

Documentation

Contains the SynthAssist Software User Guide.

Help

Contains the screen accessible Help files. Press F1 to access Help.

Log

Contains the new Log files created via SynthAssist Software 3.1 or copied from Version 3.0.

Macintosh Files

Contains the Macintosh files, *e.g.,* chemistries, runs, and sequences that you wish to convert to the PC format and use with SynthAssist Software 3.1. This folder must also contain the Macintosh "SynthAssist Directory" used to create the sequences, chemistries, and runs you wish to convert. See Chapter 3, "Converting Macintosh® Files," for more information.

Monitor Bmps

Contains the Monitor displays that have been converted to a bmp file. See Chapter 6, "Setting Up a Peptide Run," for directions on saving monitor traces (UV or conductivity) as bmp files.

Run

Contains the run files created by SynthAssist Software 3.1, any run files copied from SynthAssist Software 3.0, and any converted Macintosh run files.

Sequence

Contains the sequence files created by SynthAssist Software 3.1, any sequence files copied from SynthAssist Software 3.0, and any converted Macintosh files.

Starting SynthAssist 3.1

To start SynthAssist 3.1:

1. Double-click the desktop icon

or use the Start menu:
Start > Programs > Applied Biosystems > **SynthAssist 3.1**.

Converting Macintosh® Files 3

This chapter covers:

Overview

Introduction

SynthAssist® Software Version 3.1 allows you to convert your existing SynthAssist Software Version 2.0 Macintosh® files into a Microsoft Windows® compatible format. You can convert SynthAssist Software 2.0 Dictionary, Chemistry, Run, and Sequence files. You do not have to manually re-enter any files that have been previously developed or customized.

Before Converting Files

Before converting SynthAssist Software 2.0 files, you must copy the Macintosh files into specific SynthAssist Software 3.1 folders on your Windows platform computer. Use Table 3-1 to select the correct folder.

Table 3-1 Macintosh Files and Their Corresponding SynthAssist 3.1 Folders

Copy This Macintosh File Type...	Into This SynthAssist 3.1 Folder...
Dictionary	Macintosh Files
Chemistry	Mac Chemistry
Run	Mac Run
Sequences	Mac Sequence

Note: SynthAssist Software 3.1 installs a copy of the standard Macintosh 2.0 dictionary in the Macintosh Files folder. The standard v2.0 dictionary can be used to convert Macintosh files unless new residues or blocking groups were defined for the sequences that are to be converted. In this case the v2.0 dictionary containing the new definitions must be copied to the Macintosh Files folder in SynthAssist Software 3.1. Overwrite the standard v2.0 dictionary previously installed or remove the standard v2.0 dictionary to a PC floppy.

Transferring Files

The simplest procedure to transfer files from a Macintosh to a PC is to copy the Macintosh files onto a PC diskette. Macintosh computers running OS 7.0 or higher can R/W directly to a PC floppy via PC Exchange.

Problems Copying Files

Problems can occur when copying SynthAssist Software 2.0 Macintosh files onto a PC diskette on a Macintosh computer running an operating system earlier than OS 8.

To copy the Macintosh files successfully onto a PC diskette, ensure PC Exchange is running on the Macintosh computer.

File Name Changes

The Macintosh file names can change when the diskette is viewed on a PC. The following figures show the names of three files that were copied to a PC diskette on a Macintosh computer running OS 7.5.5. Figure 3-1 displays the file names viewed on the Macintosh computer. Figure 3-2 displays the file names – on the same diskette – viewed on a PC running the Windows® 2000 operating system. After the Mac files have been copied to the Macintosh Files folder in SynthAssist 3.1, correct the file names as appropriate. Delete any special Macintosh characters, such as "Ω" symbols.

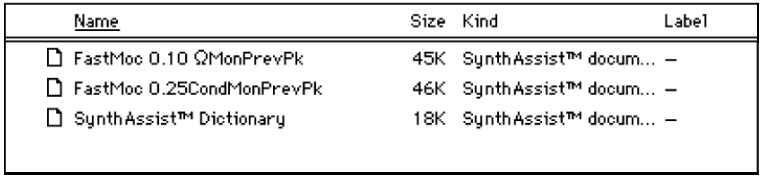


Figure 3-1 Names of Copied Files as Viewed from a Macintosh Computer

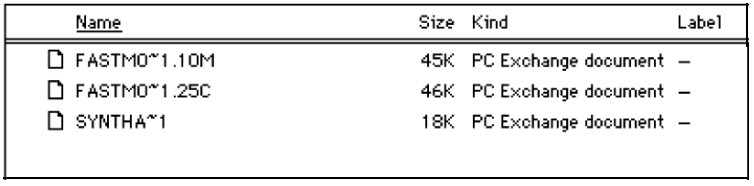


Figure 3-2 Names of Copied Files as Viewed from a PC

Converting a Macintosh Dictionary File

It is not necessary to convert a Macintosh dictionary file to the PC format unless the file contains the definition of special residues or blocking groups for v2.0 sequences that are to be carried forward into v3.1. If a v2.0 "SynthAssist™ Dictionary" is converted into PC format, it will overwrite the standard "AB433Dictionary" file that has been installed in the Common folder of v3.1.

To convert a Macintosh dictionary file:

1. Select **Convert Macintosh Files** in the Data Converter.

   The Data Converter dialog box opens with Dictionary selected by default in the Select File Type group box (Figure 3-3).

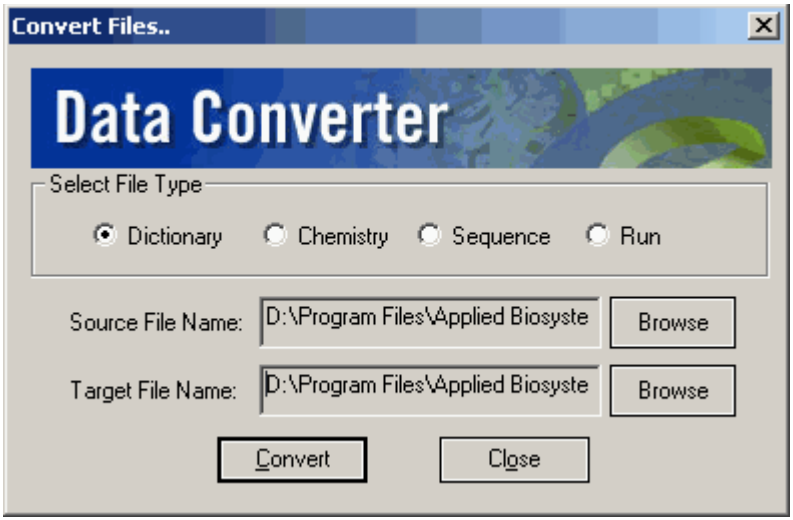


Figure 3-3 The Data Converter Dialog Box

Place the Macintosh dictionary to be converted into the Macintosh Files folder. Either overwrite the existing Macintosh dictionary that has been installed or remove the standard Macintosh dictionary to a PC floppy. Never allow more than one v2.0 dictionary file to be present in the Macintosh Files folder.

IMPORTANT! If the Macintosh dictionary file containing the special residue definitions has become corrupted, it will fail the conversion process. Use the standard PC dictionary to re-define the special residues and re-create the sequence.

2. Click **Browse** to locate the "SynthAssist™ Dictionary" installed in the Macintosh Files folder (Figure 3-4).

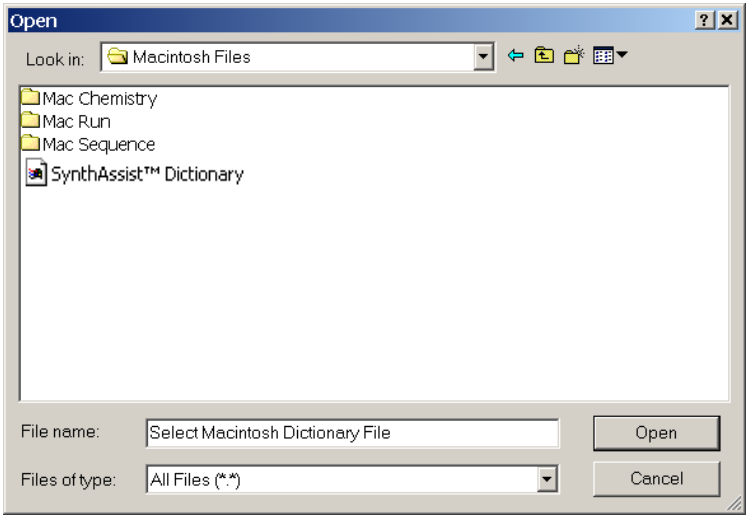


Figure 3-4 Open Dialog Box

3. Select **SynthAssist™ Dictionary** in the desired directory, then click **Open**.

   IMPORTANT! Do not change the Target File Name default directory ([Installation Directory]\Applied Biosystems\SynthAssist3.1\Common\AB433Dictionary.dic).

4. Click **Convert**. If the software prompts you to replace any existing dictionary with the same name, click **OK**.

   A message showing File Converted Successfully is displayed.

5. Click **OK**.

Converting a Macintosh Chemistry File

To convert a Macintosh Chemistry file:

1. Select **Chemistry** in the Select File Type group box of the Data Converter dialog box.
2. Click **Browse** to search for the Source File Name. A dialog box displays by default the Macintosh Chemistry files in [Installation Directory] \Applied Biosystems\SynthAssist3.1\ Macintosh Files\Mac Chemistry (Figure 3-5).

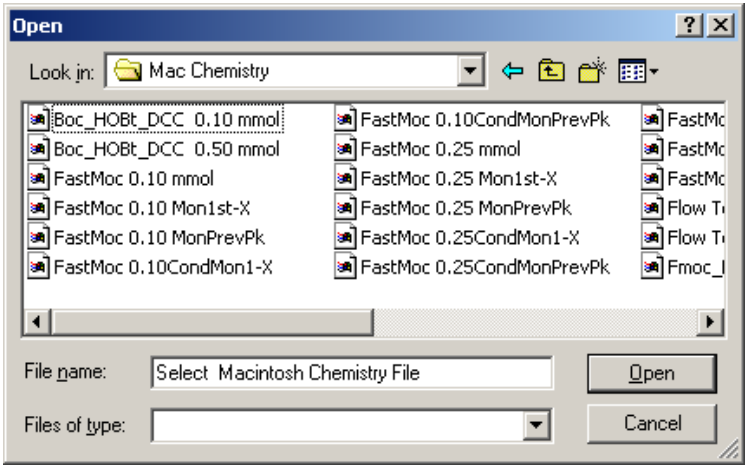


Figure 3-5 Open Mac Chemistry Dialog Box

3. Select a Chemistry file, then click **Open**.
4. Click **Browse** to search for the Target file name.

   A dialog box displays any previously converted chemistry files in [Installation Directory]\Applied Biosystems\SynthAssist3.1\ Chemistry (Figure 3-6).

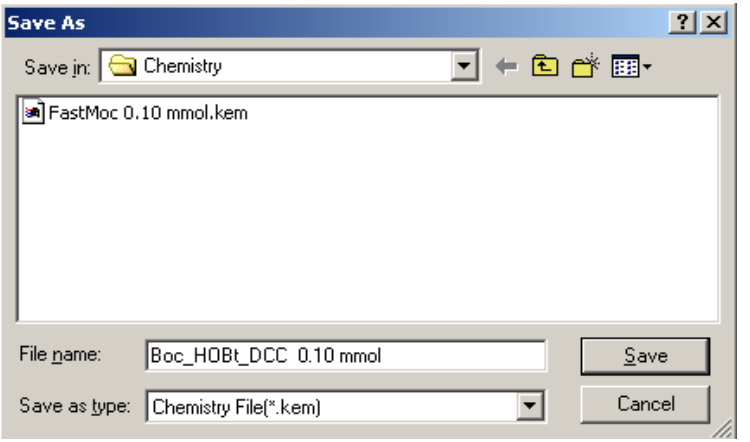


Figure 3-6 Save As Dialog Box

By default the file name in the File name box corresponds to the unconverted (Macintosh) chemistry file. You can change this file name, but do not change the default directory.

5. Click **Save**.
6. Click **Convert**.
7. Select **SynthAssist Dictionary** in the desired directory, click **Open,** then click **Yes** to confirm the chemistry file as the default.

   IMPORTANT! Saving a chemistry file as default enables the file to be write-protected. Click **No** if the Chemistry file is to be modified. If you need to make changes to a write-protected file, you must save the file with a different file name using Save As.
8. Click **OK** to confirm that the file converted successfully**.**

Converting a Macintosh Sequence File

To convert a Macintosh Sequence file:

1. Select **Sequence** in the Select File Type group box of the Data Converter dialog box.
2. Click **Browse** to search for the Source File Name.

   A dialog box displays the Macintosh Sequence files in [Installation Directory] Applied Biosystems\SynthAssist3.1\ Macintosh Files\Mac Sequence (Figure 3-7).

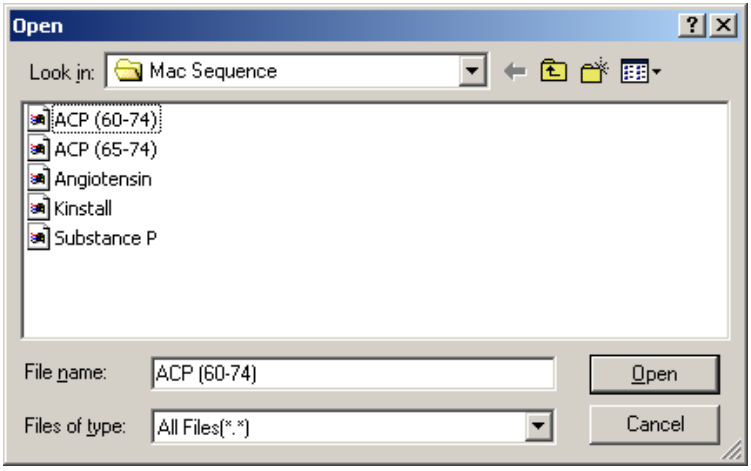


Figure 3-7 Open Mac Sequence Dialog Box

3. Select a Sequence file, then click **Open**.
4. Click **Browse** to search for the Target File Name.

   A file Save As dialog box displays any previously converted sequence files in [Installation Directory]\Applied Biosystems\ SynthAssist3.1\Sequence.

   IMPORTANT! By default, the file name in the File name box corresponds to the unconverted (Macintosh) sequence file. If required, you can change this file name, but do not change the default directory.

5. Click **Save**.
6. Click **Convert**.

7. Select **SynthAssist Dictionary** in the desired directory, click **Open**, then click **OK** to confirm successful file conversion.

Converting a Macintosh Run File

To convert a Macintosh Run file:

1. Select **Run** in the Select File Type group box of the Data Converter dialog box.
2. Click **Browse** to search for the Source File Name.

   A dialog box (Figure 3-8) displays the Macintosh Run files under [Installation Directory]\Applied Biosystems\SynthAssist3.1\Macintosh Files\Mac Run.

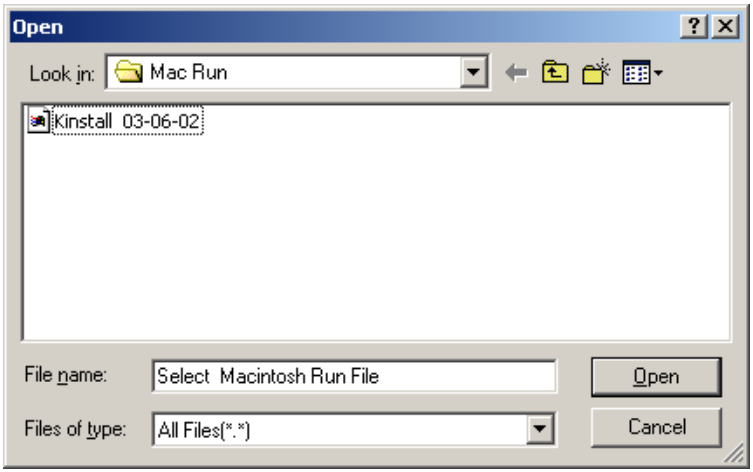


Figure 3-8 Mac Run Open Dialog Box

3. Select a Run file, then click **Open**.
4. Click **Browse** to search for the Target File Name.

   A dialog box displays any previously converted Run files in [Installation Directory]\Applied Biosystems\SynthAssist3.1\Run (Figure 3-9).

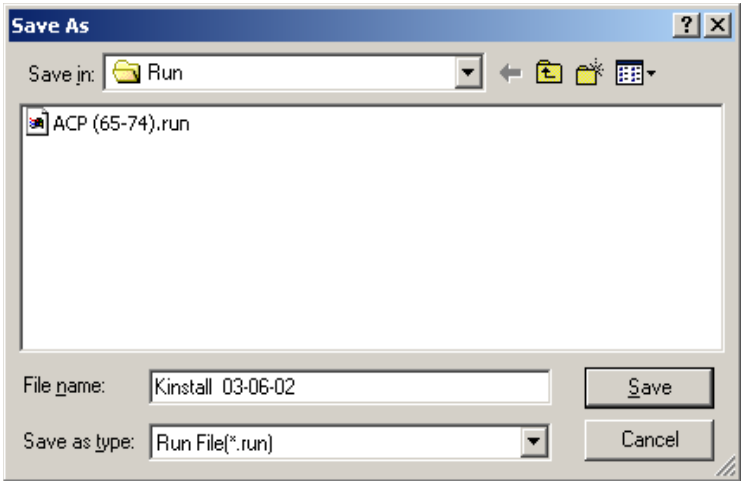


Figure 3-9 Run Save As Dialog Box

5. Click **Save**.

   IMPORTANT! By default the file name in the File name edit box corresponds to the unconverted (Macintosh) Run file. If required, you can change this file name, but do not change the default directory.

6. Click **Convert**.

7. Select **SynthAssist Dictionary** in the desired directory, then click **Open**.

   The Provide Converted Chemistry and Sequence File Name dialog box opens.

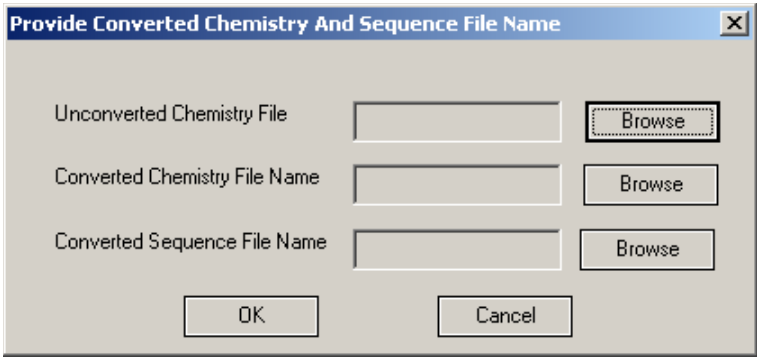


Figure 3-10 Provide Converted Chemistry and Sequence File Name Dialog Box

8. Click **Browse** to search for the Unconverted Chemistry file.

   A dialog box displays the Macintosh Chemistry files under [Installation Directory]Applied Biosystems\SynthAssist3.1\ Macintosh Files\Mac Chemistry.

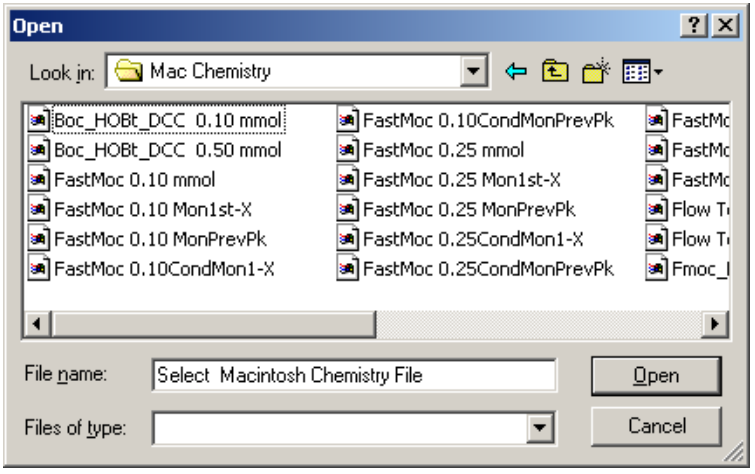


Figure 3-11 Mac Chemistry Open Dialog Box

9. Select the Chemistry file associated with the Run, then click **Open**.

10. Click **Browse** to search for the Converted Chemistry File Name, select the converted Chemistry File associated with the above Run, then click **Open**.
11. Click **Browse** to search for the Converted Sequence File Name, select the converted Sequence File associated with the above Run, then click **Open**.
12. Click **OK** to confirm successful file conversion.

Communications 4

This chapter covers:

Communications: PC to ABI 433A Peptide Synthesizer

Communications between the PC and 433A Synthesizer must be established before chemistry or run files can be sent to the synthesizer. The steps of the software connection process are outlined below. The hardware connection is a cable from Com1 of the PC to the 433A Synthesizer Port A.

Enabling Communication

Communication between the PC and the 433A Synthesizer is established via Synthesizer/Connect from the Main Menu. A Connect to Synthesizer window opens and requires three selections. Click **Communications Enabled**. Click the Select Port tab to choose Com1. Lastly, click **OK**. A status message at the bottom-right of the screen displays the communication status: Not Connected/Connected.

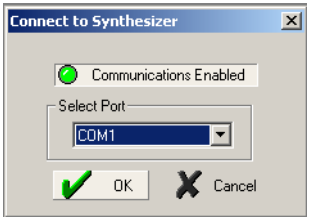


Figure 4-1 Connect to Synthesizer

Set Clock

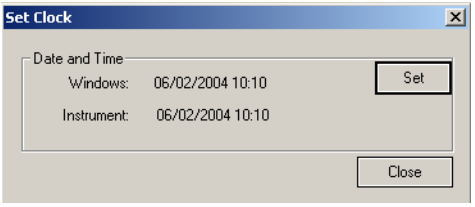


Figure 4-2 Set Clock

From the main menu select **Synthesizer/Set Clock.** Click **Set** to change the 433A Synthesizer date and time if they differ from the PC. If communication has not been enabled previously, the Connect to Synthesizer window will open to enable communication before the Set Clock option can continue.

Auto-Save

From the main menu click **File/Auto-Save** to open the screen below.

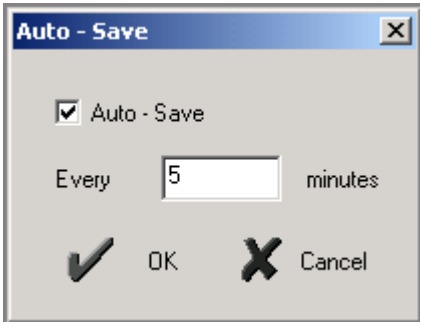


Figure 4-3 Auto-Save

The Auto-Save checkbox is pre-selected and set to 5 minutes by default. Clicking **Cancel** closes the AutoSave dialog box, leaving the previous settings in effect. Clicking **OK** closes the AutoSave dialog box and implements the settings displayed.

Sending a Flow Test Chemistry

Twenty-one predefined (conductivity monitoring) Chemistry files are provided with SynthAssist 3.1 under the **C:\ Program Files\ Applied Biosystems\ SynthAssist3.1\ Chemistry** directory. In addition, two subfolders of special chemistry files are now included:

- the 3 mL Original Chemistry (tBoc/PNA and conductivity)
- the S200 UV Chemistry.

See the SynthAssist Software v3.1 Release Notes for a further explanation of the chemistry files in these two folders.

By default the installed chemistry files are locked or read-only. A pre-defined Chemistry file must be “Saved As” an unlocked Chemistry file before any changes to the chemistry can be made, such as, modifying modules and cycles, or using the Functions page to select valves and define a user function.

Click **File/Open** from the SynthAssist Software main menu to display the screen below.

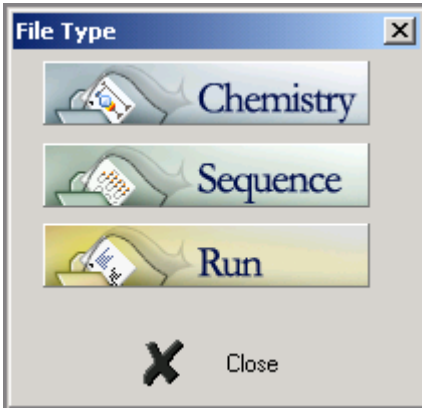


Figure 4-4 The File/Open Screen

Selecting **Chemistry** will open the Chemistry folder to display the screen below.

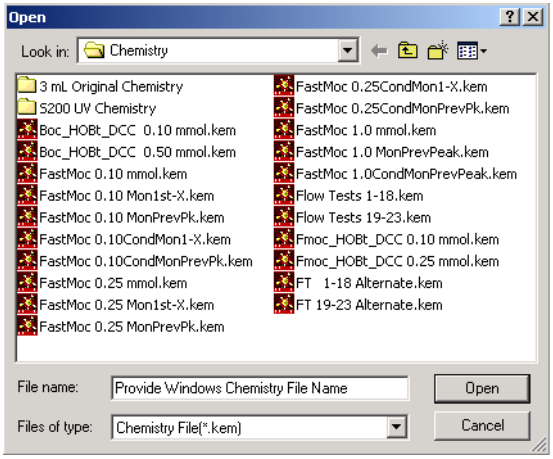


Figure 4-5 The Chemistry Folders and Files in SynthAssist 3.1

Double-click on **Flow Tests 1-18.kem** to open the Chemistry file below.

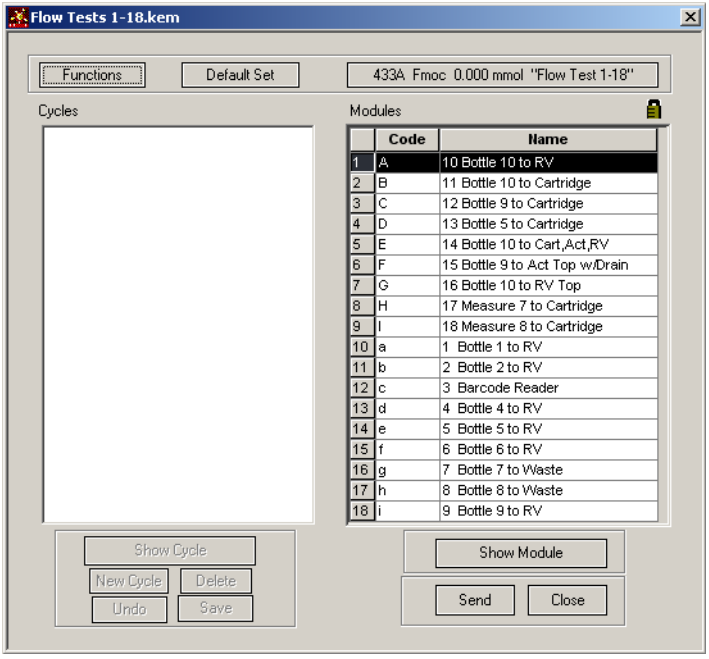


Figure 4-6 Flow Tests 1-18

Click **Send** to open the Send Dialog box.

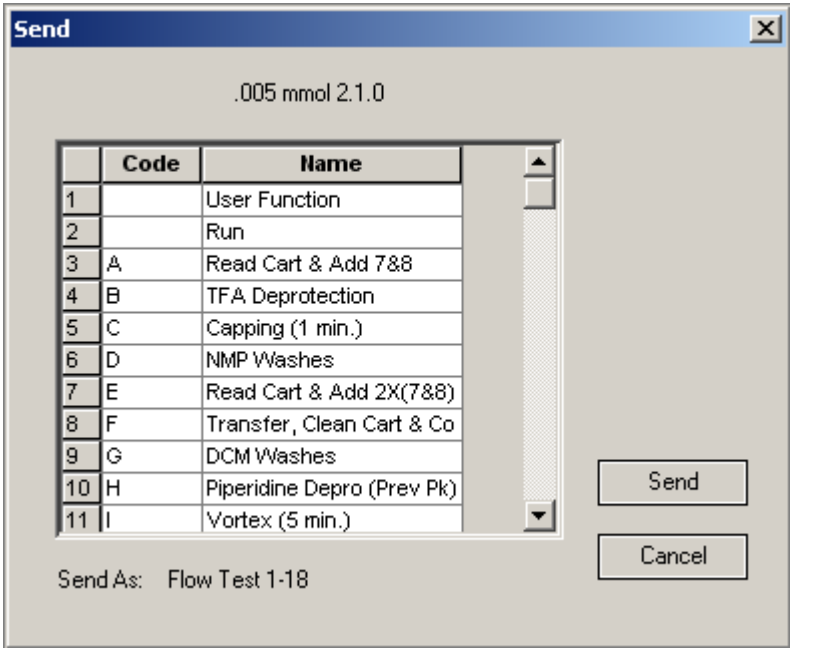


Figure 4-7 The Send Dialog Box

Click **Send** to start the transmission process. The Send Progress box fills as the files are sent over.

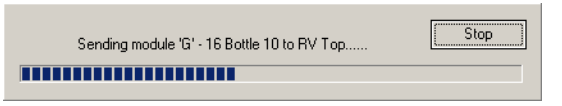


Figure 4-8 The Send Progress Box

Note: The synthesizer acknowledges the transmission by chirping each time it receives a module.

Running Flow Tests

Consult the ABI 433A Peptide Synthesizer User Guide for details on the Flow Tests and specifications required to verify the proper operation of the instrument.

The Sequence 5

This chapter covers:

Specifying the Sequence of Amino Acids

Creating a Sequence

To create an amino acid sequence:

1. Select **File** > **New**.

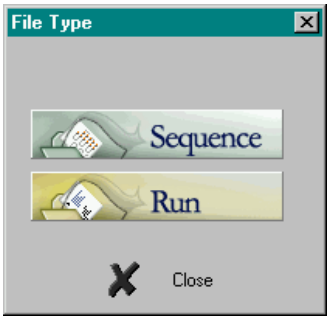


Figure 5-1 File Type Dialog Box

2. In the File Type dialog box (Figure 5-1), click **Sequence**. The Sequence screen opens along with the AA palette screen (Figure 5-2).

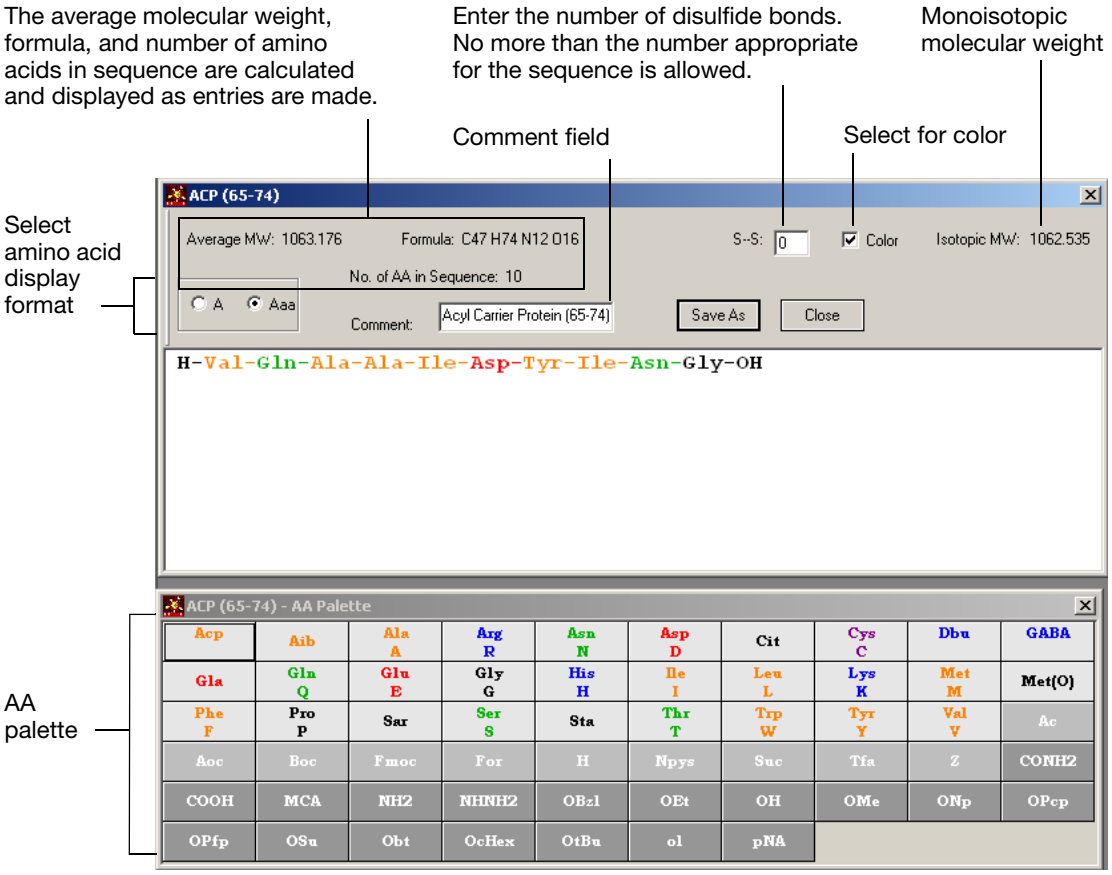


Figure 5-2 Sequence Screen and AA Palette

3. At the sequence screen enter the amino acid by clicking the amino acid keys in the AA palette, or by typing the one-letter codes.

   The chemical formula, number of amino acids in the sequence, average molecular weight, and the monoisotopic molecular weight are automatically calculated and displayed on the Sequence screen as you make the entries.

4. Highlight the C- or N-terminus (OH or H) of the Sequence in the Sequence screen. The palette changes to a selection of C- or N-termini.

5. Click **Save As**, then name the sequence.

   The name appears in the title bar of the Sequence screen.

6. Select a button on the left above the Sequence to specify the format of the amino acid sequence.

7. Select **A** to display the sequence using the one-letter format (Figure 5-3).

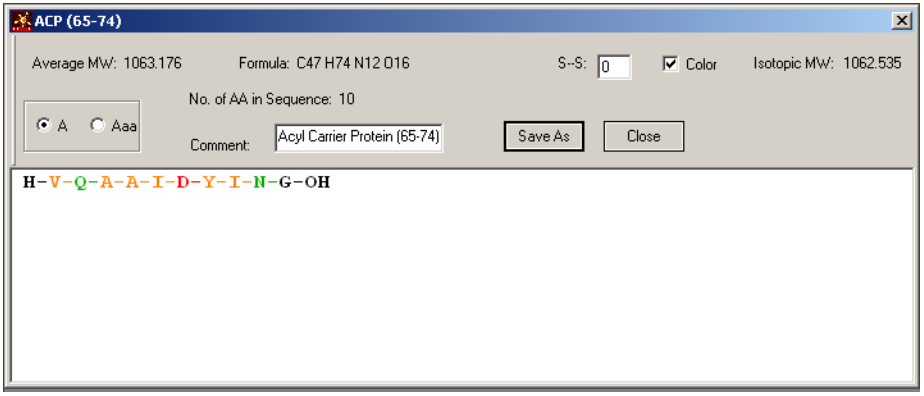


Figure 5-3 One-Letter Sequence Format

8. Select **Aaa** to display the sequence using the three-letter format (Figure 5-4).

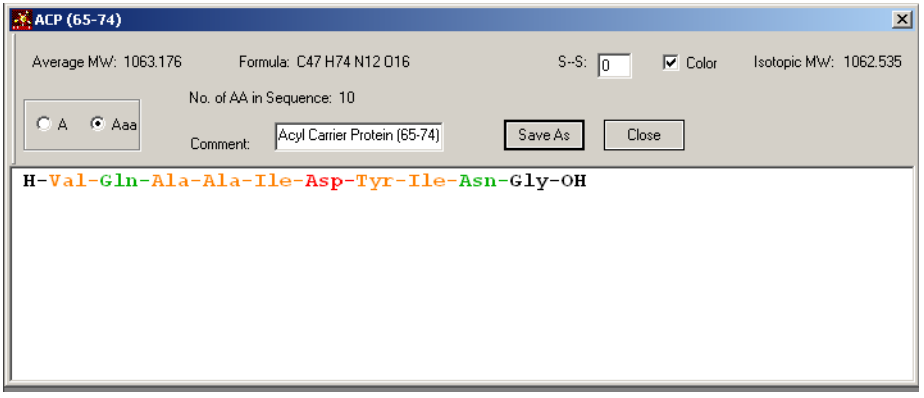


Figure 5-4 Three-Letter Sequence Format

9. Select **File > Print Preview** to view the sequence as it will appear in printed form.
10. Select **File > Print** (or press **Ctrl+P**) to print the sequence screen.
11. When you finish, select **File > Save As**, then type in the name of the sequence.
12. Click **Save**. By default the new sequence will be saved in the Sequence folder.

Undefined Residues and Termini

Accessing a Sequence

To access an existing sequence:

1. Select **File > Open**.
2. Click **Sequence**.
3. Locate the sequence file of interest, select it, then click **Open.**

About Undefined Residues

When an existing sequence is opened, SynthAssist® Software Version 3.1 checks each residue and each terminus in the sequence for its definition in the current dictionary. If a residue in the sequence is not defined, *e.g.,* the sequence was copied into your SynthAssist software from another site with a different dictionary, the following message appears (Figure 5-5).

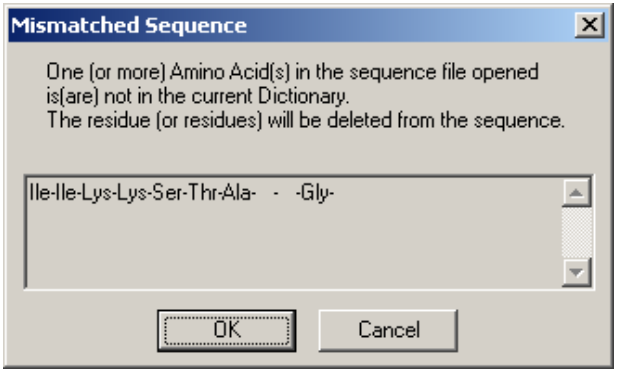


Figure 5-5 Mismatched Sequence Message Box

The dialog box displays the sequence with a blank space substituted for the undefined residue(s). You can click **OK** to open the deleted sequence or click **Cancel**.

About Undefined Termini

Unidentified N-Terminus

If a terminus in the sequence is not defined, *e.g.,* the sequence was copied into your SynthAssist from another site with a different dictionary, the following message appears (Figure 5-6).

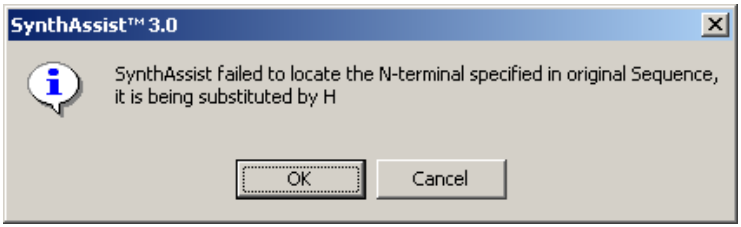


Figure 5-6 N-Terminal Substitution Message Box

When the sequence is opened, the new N-terminus is displayed as "H."

Unidentified C-Terminus

For an undefined C-terminus, the following message appears (Figure 5-7).

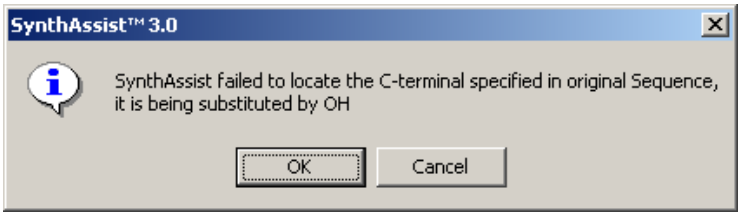


Figure 5-7 C-Terminal Substitution Message Box

When the sequence is opened, the new C-terminus is displayed as "OH."

Changing the Peptide Sequence

Deleting an Amino Acid

To delete an amino acid, do one of the following:

Method A:

1. Place the cursor to the right of the amino acid you want to delete.
2. Press the backspace key (◄—) or the Delete key. The amino acid is deleted from the sequence.

Method B:

1. Select the amino acids you want to remove by dragging the cursor through them.
2. Select **Edit > Cut**, or press the **Delete** key. The selected amino acid(s) are deleted from the sequence.
3. Select **File > Save As** and provide a new name for the sequence.

Adding an Amino Acid

To add an amino acid:

1. Place the cursor in the location where you want to add an amino acid.
2. Select the amino acid from the AA palette, or type the one-letter code for the amino acid on the keyboard.
3. Select **File > Save As** and provide a new name for the sequence.

Changing Amino Acids

To change amino acids:

1. Select the amino acids you want to change.
2. Delete the amino acids you want to change.
3. Enter the new amino acids.
4. Select **File > Save As** and provide a new name for the sequence.

Changing Termini

To change N-terminus or C-terminus

1. Select a terminus in the sequence. The corresponding buttons in the palette are enabled.
2. Click a new terminus compound in the Palette.

3. When you finish, select the Sequence.
4. Select **File > Save As** and provide a new name for the sequence.

Using Cut/Copy/Paste in an Amino Acid Sequence

To use copy and paste in a Sequence:

1. Open the Sequence file from which the sequence is to be copied.
2. Select a portion of the amino acid sequence, then select **Edit** > **Cut** or **Edit** > **Copy**.

   IMPORTANT! You can copy only the amino acids in a sequence but not the termini.
3. Open or create the sequence where the copied amino acids are to be pasted.
4. Place the cursor where you want to insert the sequence, then select **Edit** > **Paste**.

   IMPORTANT! Do not use Ctrl+C, Ctrl+V to copy/paste in the Comment field. To copy a part of a comment, select, then right-click the part of interest, then select **Copy**. To paste text in the Comment field, place the cursor at the right in the Comment field, right-click, then select **Paste**.

   Note: You can use Microsoft Word®, WordPad®, or Notepad® to cut/copy/paste sequences from the Sequence screen to other text editors, or vice versa. However, copying between sequences of single-letter format (A) to triple-letter format (Aaa) or vice versa is not permitted.

Deletion Sequences

Creating Deletion Sequences

The Sequence page is also useful in the search for deletion peptides that may be produced during a difficult synthesis. Removing one or more residues from the starting sequence gives the mass of a suspected deletion peptide that can be compared with mass spec data.

To create deletion sequences:

1. Open the starting sequence and copy/paste it into a new sequence.
2. Create deletions in the new sequence while the starting sequence remains displayed on the screen for comparison.

   Note: If a set of deletions in the new sequence begins to lose track of the original residues, copy/paste again from the starting sequence into the new sequence.
3. When a particular deletion sequence matches a mass spec peak, use **File>Save As** to name the deletion peptide.

The Run 6

This chapter covers:

Setting Up a Peptide Run

Starting a New Run

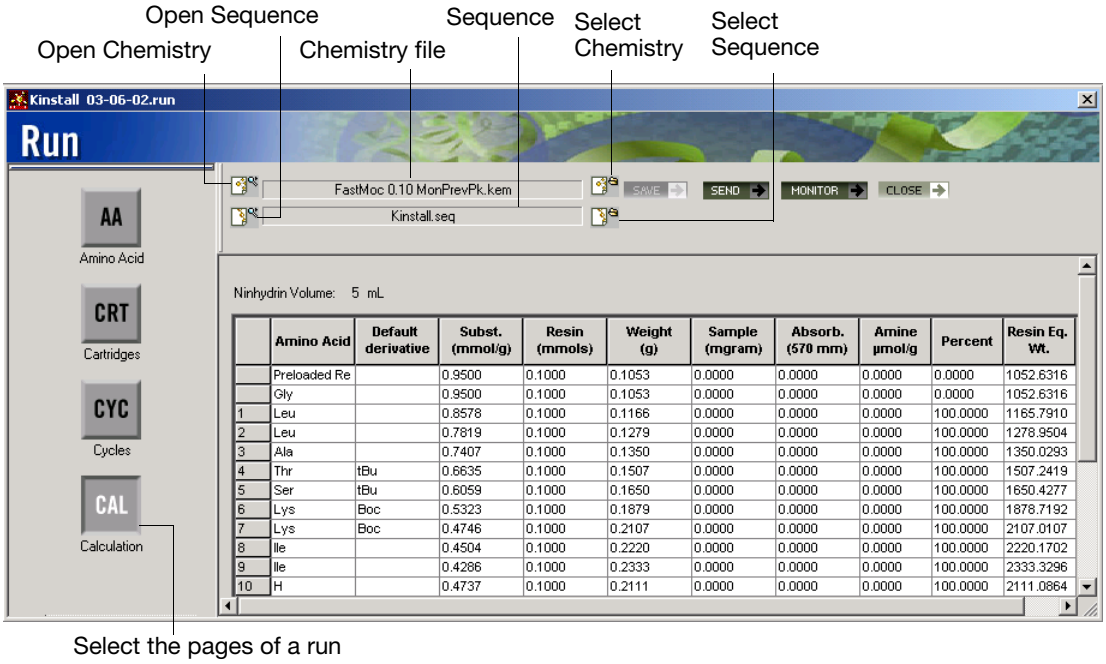
To start a new run:

1. Select **File** > **New**, or press **Ctrl+N**.

   The File Type dialog box opens.

Figure 6-1 File Type Dialog Box

2. Click **Run**. The Run screen opens with the Calculation view (Figure 6-2).



	Amino Acid	Default derivative	Subst. (mmol/g)	Resin (mmols)	Weight (g)	Sample (mgram)	Absorb. (570 nm)	Amine µmol/g	Percent	Resin Eq. Wt.
	Preloaded Re		0.9500	0.1000	0.1053	0.0000	0.0000	0.0000	0.0000	1052.6316
	Gly		0.9500	0.1000	0.1053	0.0000	0.0000	0.0000	0.0000	1052.6316
1	Leu		0.8578	0.1000	0.1166	0.0000	0.0000	0.0000	100.0000	1165.7910
2	Leu		0.7819	0.1000	0.1279	0.0000	0.0000	0.0000	100.0000	1278.9504
3	Ala		0.7407	0.1000	0.1350	0.0000	0.0000	0.0000	100.0000	1350.0293
4	Thr	tBu	0.6635	0.1000	0.1507	0.0000	0.0000	0.0000	100.0000	1507.2419
5	Ser	tBu	0.6059	0.1000	0.1650	0.0000	0.0000	0.0000	100.0000	1650.4277
6	Lys	Boc	0.5323	0.1000	0.1879	0.0000	0.0000	0.0000	100.0000	1878.7192
7	Lys	Boc	0.4746	0.1000	0.2107	0.0000	0.0000	0.0000	100.0000	2107.0107
8	Ile		0.4504	0.1000	0.2220	0.0000	0.0000	0.0000	100.0000	2220.1702
9	Ile		0.4286	0.1000	0.2333	0.0000	0.0000	0.0000	100.0000	2333.3296
10	H		0.4737	0.1000	0.2111	0.0000	0.0000	0.0000	100.0000	2111.0864

Figure 6-2 Run Screen

Selecting a Chemistry

You can select a Chemistry file different from the one displayed in the Chemistry file box.

Note: By default a new run always displays the Chemistry previously sent to the synthesizer.

To select a chemistry file:

1. Click the Chemistry file selection icon.

   A File Open dialog box opens, displaying the Chemistry folder (Figure 6-3).

2. Select the Chemistry file of interest, then click **Open**.

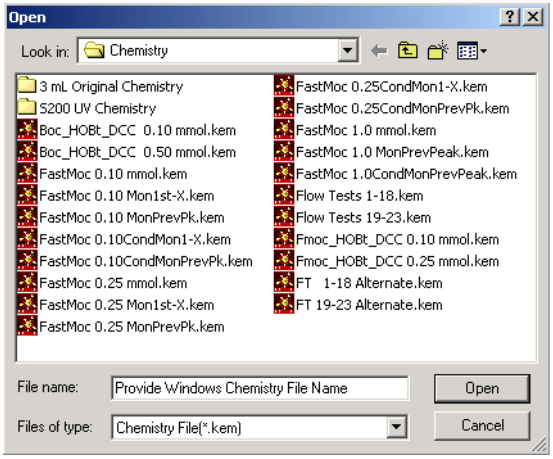


Figure 6-3 Selecting a Chemistry File

Selecting the Run Sequence

To select the run sequence:

Note: Creating a new run requires selecting a new sequence.

1. Click the **Select Sequence icon** on the Run page to open the Sequence folder.
2. Double-click the Sequence file of interest, or highlight the file and click **Open**.
3. The residues of the sequence fill-in the Run pages. The sequence name appears in the sequence box of the Run.

Peptide Resin Calculations

Note: You can print any of the four views in a Run file by selecting **File** > **Print** for the current screen.

Selecting the Type of Resin, Substitution, and Chemistry Scale

To select the type of resin:

1. Click the resin type cell, then select the desired resin type from the drop-down list (Figure 6-4).

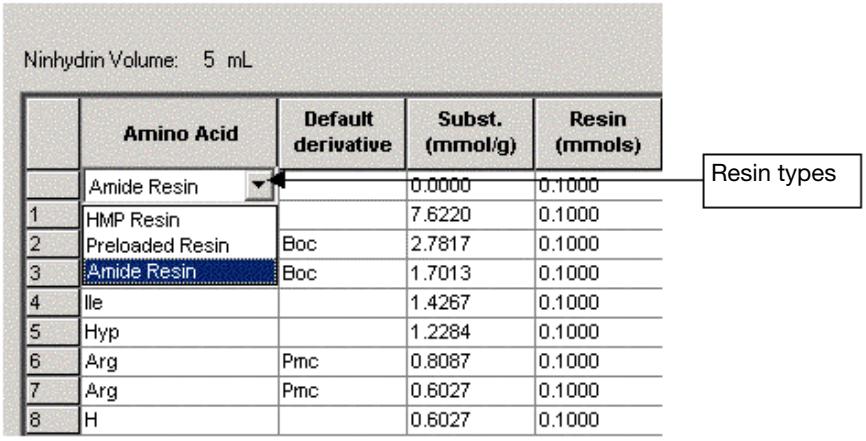


Figure 6-4 Selecting Resin Types

For FastMoc or Fmoc chemistries, the resin types are Amide Resin, HMP Resin, and Preloaded Resin, as predefined in the Dictionary file. Additional resins can be defined in the Dictionary.

2. Press **Tab** to refresh the view.
3. Click the substitution entry (the first field in the Subst. column).
4. Enter the Substitution value.
5. Press **Tab** to refresh the calculation.
6. Click the **Resin** (mmols) field, then enter the mmol scale if necessary.

   Note: The Resin (mmols) field is carried over from the selected Chemistry.

7. Press **Tab** to refresh the calculation.

   Note: The amount of resin needed is indicated in the Weight (g) field. If you change the Resin Substitution value, the Scale, or the Resin weight, SynthAssist software calculates new values for the other two parameters.

Changing Protecting Groups

To change protecting groups:

1. Click the default derivative cell for the amino acid you want to change (Figure 6-5).
2. From the drop-down list, select a protecting group, then press **Tab** to refresh the view.

   Note: The list of protecting groups is defined in the Dictionary for each amino acid.

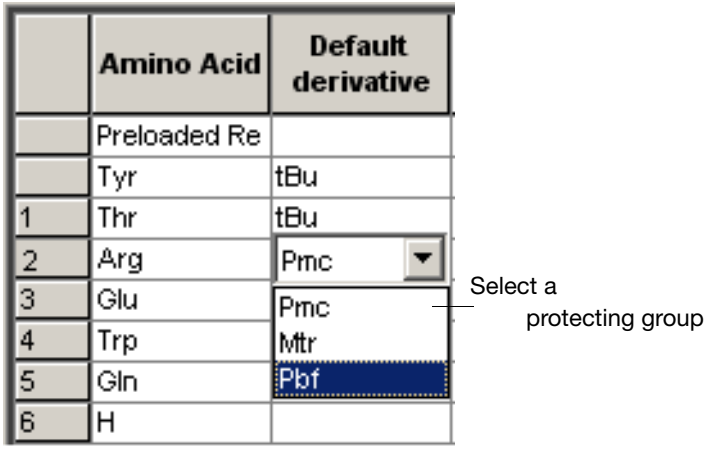


Figure 6-5 Selecting a Protecting Group

Changing the Run Cycles

To change the run cycles:

1. Click **Cycles** in the Run view selection panel (Figure 6-6) of the Run Window.

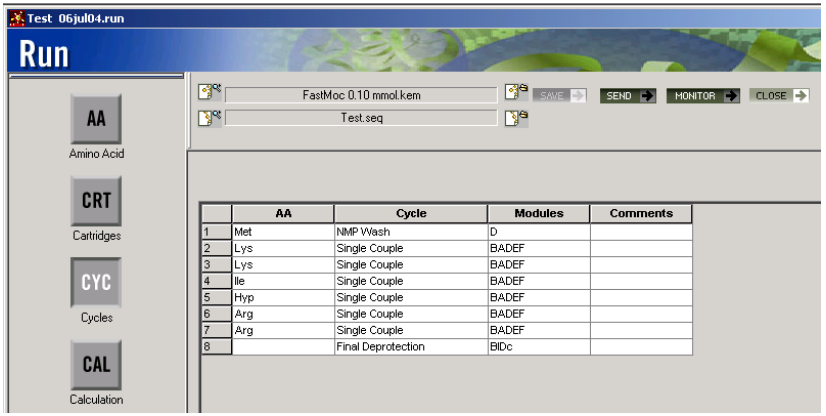


Figure 6-6 Cycle Selection Panel

2. Click the cycle cell for the cycle you want to change (Figure 6-7).
3. In the drop-down list, select the cycle you want to change.

Use the pop-up field to override the cycle selected for an amino acid

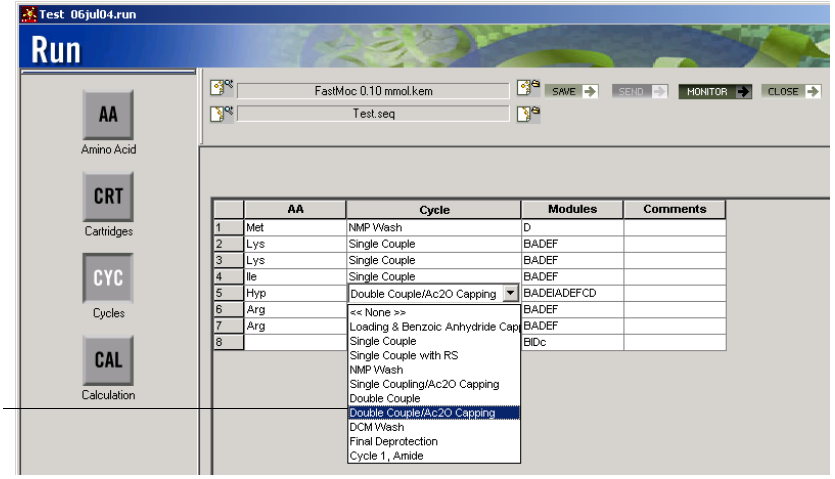


Figure 6-7 Changing the Default Cycle

Determining Different Amino Acids

To determine the number of different amino acids in the peptide:

1. Click **Amino Acid** in the Run view selection panel.

   A list of the different amino acids and the number of amino acids in the peptide (in numeric and bar-graph format) appear in the screen (Figure 6-8).

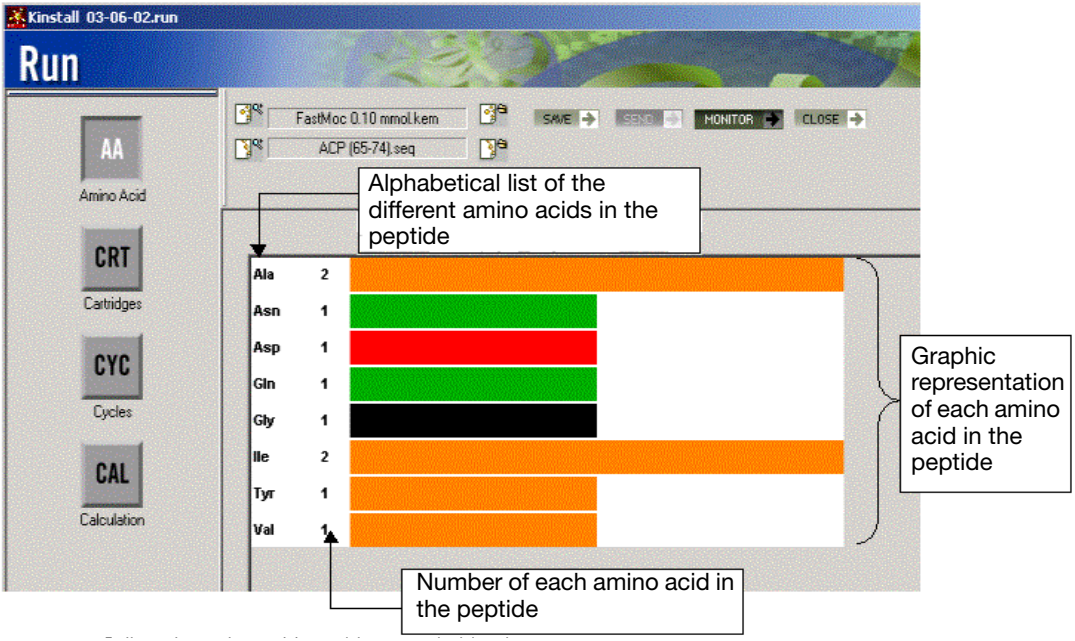


Figure 6-8 Amino Acid in the Run View

Determining the Order of Cartridges

To determine the order of amino acid cartridges needed for the run:

1. Click **Cartridges** in the Run view selection panel.

   An amino acid cartridge template is displayed (Figure 6-9).

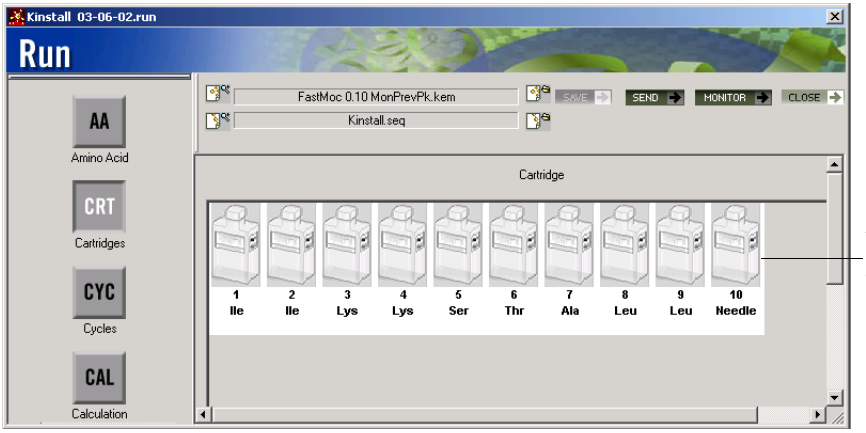


Figure 6-9 Amino Acid Cartridge Template

2. Select **File** > **Print**, or press **Ctrl+P** to print the template.
3. Lay out the amino acid cartridges on the template, then load them onto the synthesizer.

   IMPORTANT! Load the amino acid cartridges from the C-terminal end.

   IMPORTANT! When viewed in the 433A guideway, the N-terminal cartridge is on the left. The first C-terminal cartridge to be activated is on the right and is adjacent to the Needle cartridge. The Needle cartridge is present as a reminder to place an empty (used) cartridge under the needles at the start of every synthesis run.

Sending a Peptide Synthesis

If you have not saved your files, select **File** > **Save**, or press **Ctrl+S**, or click **Save**.

Sending the Chemistry File to the Synthesizer

To send the chemistry file to the synthesizer:

IMPORTANT! If you have not reset the synthesizer or not changed user functions or modules, or if you are not changing chemistry files, you can skip the following steps.

1. Click the chemistry file icon in the Run screen (Figure 6-2). The Chemistry screen opens (Figure 6-10).

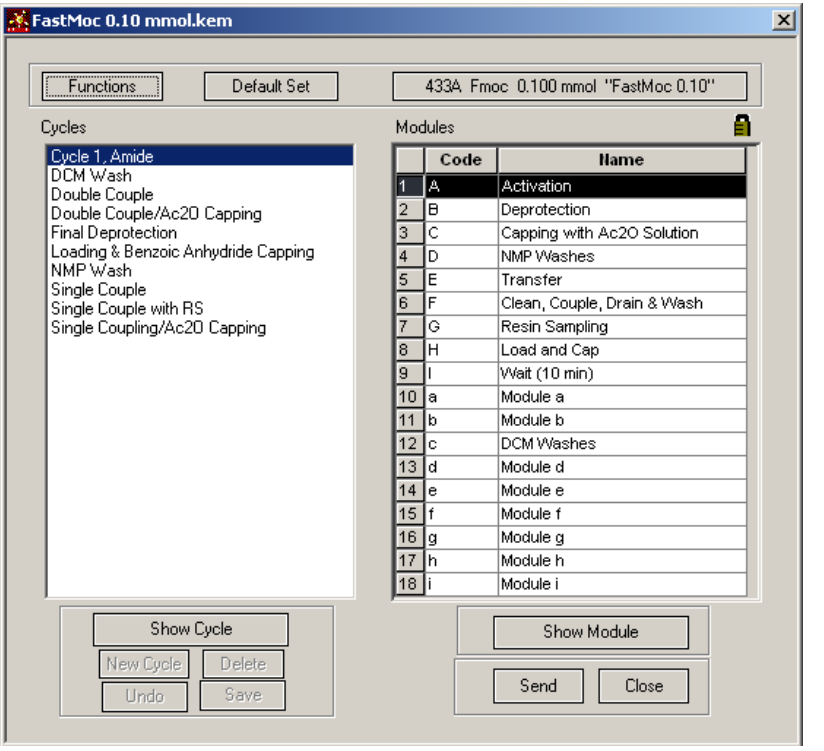


Figure 6-10 Chemistry Screen

2. Select **Synthesizer > Send**.

   If you are not already connected to the synthesizer, the Connect to Synthesizer dialog box opens (See Chapter 4, Figure 4-1).

Sending the Peptide Run to the Synthesizer

Before starting a synthesis, you must send the peptide run to the ABI 433A Peptide Synthesizer to tell the SynthAssist software which run on the synthesizer is current. Before you send a run, you must first save it. When you send the run to the synthesizer, the cartridge list is also sent.

To send the peptide run to the synthesizer.

1. Select **Synthesizer** > **Send**.

   If there is a chemistry mismatch, for example, if FastMoc 0.10 is loaded on the synthesizer, but the PC is using Boc_HOBt_DCC 0.10 mmol chemistry, the message shown in Figure 6-11 is displayed.

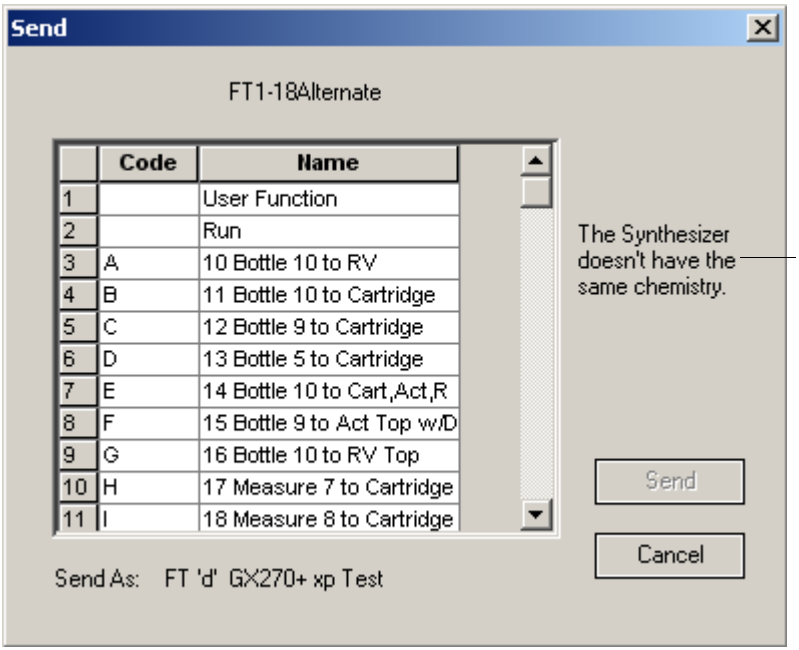


Figure 6-11 Chemistry Conflict Message

2. To correct the condition, open the Chemistry screen, select **Synthesizer** > **Send**, then click **Send** in the Communications screen.

Starting the Peptide Synthesizer

To start the peptide synthesizer:

1. On the keyboard of the peptide synthesizer, press the **cycle monitor** soft key.
2. Respond to the questions on the synthesizer screen, then press **begin**.

Viewing the Lab Screen

To view the current status of the run

1. Select **Common** > **Lab**.

   The status of the peptide synthesizer appears in the SynthAssist Lab screen (Figure 6-12).

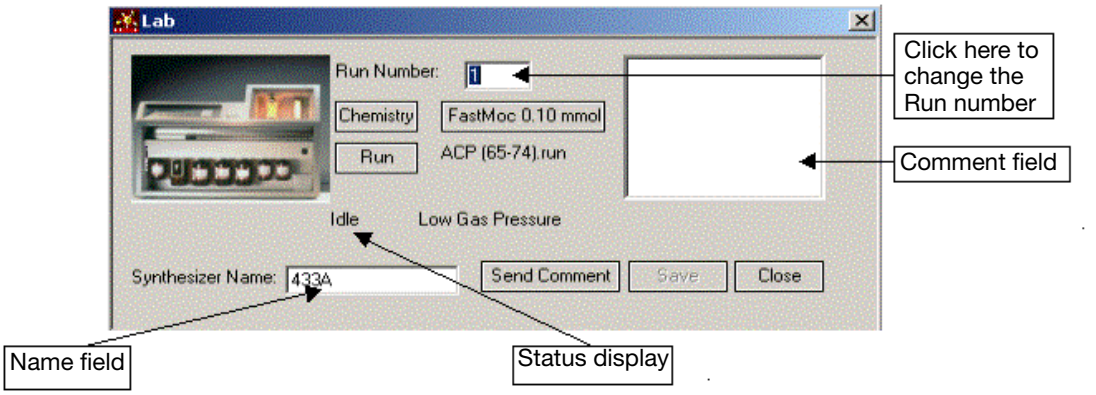


Figure 6-12 Lab Screen

2. Type the name of the peptide synthesizer in the Synthesizer Name field.

   Note: The run number increments automatically when a run is sent to the synthesizer.

3. Enter your comment in the Comment field, then click **Send Comment** to send the comment to the Log.

   Note: If your comment is very long, break it into multiple sentences, where each sentence starts with a new line. Press the **Enter** key each time you want to create a new line in the comment field. Multiple sentences in the Comment field appear as separate log messages in SynthAssist Log.

   Note: If you send a Chemistry file to the synthesizer, its name is displayed in the Lab screen. When a new run is created, it uses the Chemistry file displayed in the Lab screen. If the Chemistry file has been moved to a different folder or deleted, the Chemistry File Name field of the run becomes <<None>>. Unlike earlier versions (for Macintosh), SynthAssist 3.1 software does not use the run number as the run file name.

Viewing the Monitoring Status of the Run

To view the monitoring status of the run:

1. Click **Monitor** in the Run screen.

   The Monitor screen opens, displaying the monitoring values measured during a run. Figure 6-13 shows the conductivity deprotections typically observed for a Kinstal synthesis. The monitor window can display any values saved by Function 132, such as deprotections (conductivity or UV) or solvent conductivites measured via Flow Tests 20 and 22.

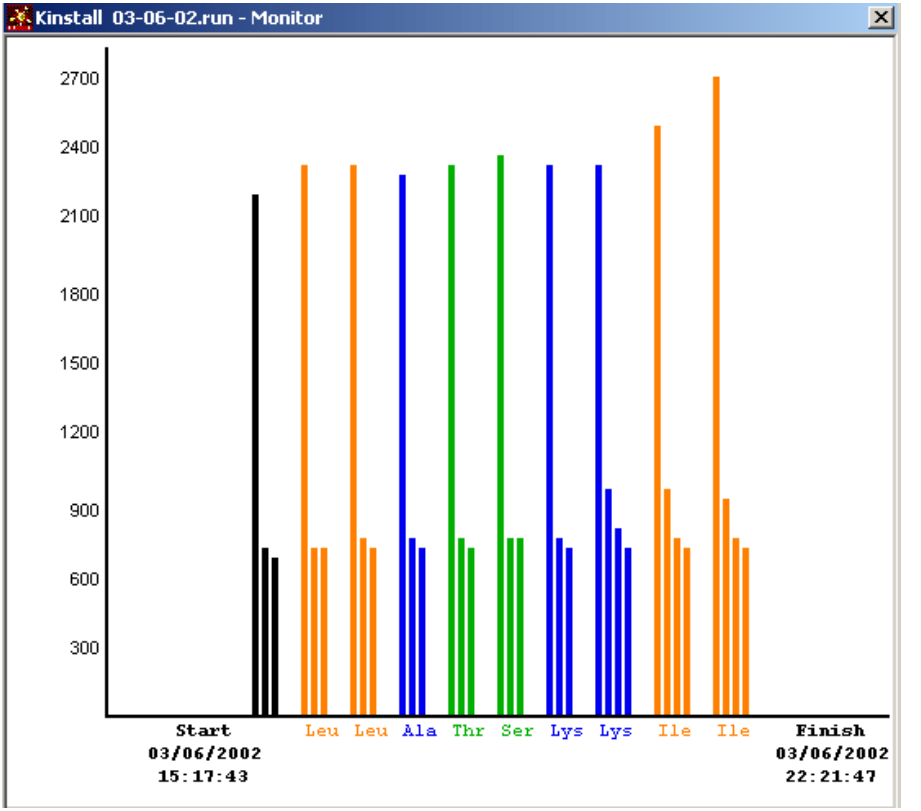


Figure 6-13 Monitor Screen

2. Print the Monitor graph by clicking **File > Print**.

3. If you want to save the Monitor graph in bitmap (.bmp) format, maximize the Monitor screen to maximize the area of the graph to save, then select **File > Save As BMP**.

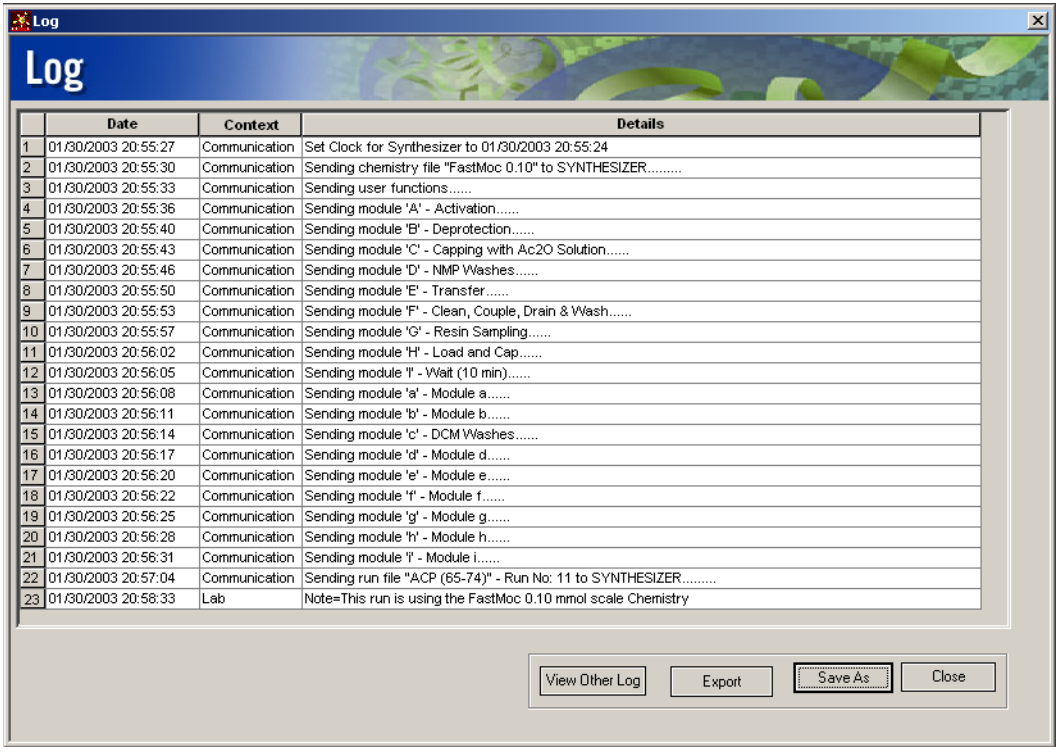
   Note: Later you can edit and/or export to other formats.

   By default the Monitor Bmp folder is displayed.

4. Name the file, then save it in the Bmp folder.

Viewing the Log

When the SynthAssist software communicates with the peptide synthesizer, the information is added to the log. To view the log, select **Common** > **Log** (Figure 6-14).



	Date	Context	Details
1	01/30/2003 20:55:27	Communication	Set Clock for Synthesizer to 01/30/2003 20:55:24
2	01/30/2003 20:55:30	Communication	Sending chemistry file "FastMoc 0.10" to SYNTHESIZER.....
3	01/30/2003 20:55:33	Communication	Sending user functions.....
4	01/30/2003 20:55:36	Communication	Sending module 'A' - Activation.....
5	01/30/2003 20:55:40	Communication	Sending module 'B' - Deprotection.....
6	01/30/2003 20:55:43	Communication	Sending module 'C' - Capping with Ac2O Solution.....
7	01/30/2003 20:55:46	Communication	Sending module 'D' - NMP Washes.....
8	01/30/2003 20:55:50	Communication	Sending module 'E' - Transfer.....
9	01/30/2003 20:55:53	Communication	Sending module 'F' - Clean, Couple, Drain & Wash.....
10	01/30/2003 20:55:57	Communication	Sending module 'G' - Resin Sampling.....
11	01/30/2003 20:56:02	Communication	Sending module 'H' - Load and Cap.....
12	01/30/2003 20:56:05	Communication	Sending module 'I' - Wait (10 min).....
13	01/30/2003 20:56:08	Communication	Sending module 'a' - Module a.....
14	01/30/2003 20:56:11	Communication	Sending module 'b' - Module b.....
15	01/30/2003 20:56:14	Communication	Sending module 'c' - DCM Washes.....
16	01/30/2003 20:56:17	Communication	Sending module 'd' - Module d.....
17	01/30/2003 20:56:20	Communication	Sending module 'e' - Module e.....
18	01/30/2003 20:56:22	Communication	Sending module 'f' - Module f.....
19	01/30/2003 20:56:25	Communication	Sending module 'g' - Module g.....
20	01/30/2003 20:56:28	Communication	Sending module 'h' - Module h.....
21	01/30/2003 20:56:31	Communication	Sending module 'i' - Module i.....
22	01/30/2003 20:57:04	Communication	Sending run file "ACP (65-74)" - Run No: 11 to SYNTHESIZER.....
23	01/30/2003 20:58:33	Lab	Note=This run is using the FastMoc 0.10 mmol scale Chemistry

Figure 6-14 Log Screen

The log has the following features:

- There is only *one* log stored in AB433Log.log file in Applied Biosystems\SynthAssist3.1\Common in the program installation folder (D:\Program Files). The log records and retains all information (for example, run, event, comments, monitoring data, all send/receive/set clock information and application errors.

- You cannot edit the log using the SynthAssist software. To edit the log, click **Export,** then enter the name of the exported file. All log data is exported in tab-delimited text format. You can view and edit the exported log data using another application such as Notepad.

IMPORTANT! Use Save As to name and save the Log that accompanies each Run carried out on the synthesizer. After saving the Log, delete it when prompted. Do not allow Logs to concatenate for multiple Runs.

- You can print the Log file by selecting **File** > **Print**.

Note: If the computer and the synthesizer are connected during a run, the monitor and Log data are automatically updated as the run progresses. If the computer and synthesizer are not connected during a run, the data is accumulated in the memory buffer of the synthesizer and will be displayed after communications are re-established.

Opening an Existing Peptide Run

To open an existing run:

1. Select the **File** > **Open**, or press **Ctrl+O**. The File Type dialog box opens.
2. Click the **Run** button. The File Open dialog box opens.
3. Select a Run file, then click **Open**.

When the Software Cannot Open a File

If SynthAssist Software 3.1 cannot open the associated sequence or chemistry files (for example, if the Run file is from another computer running SynthAssist 3.1 software), the following messages are displayed (Figure 6-15 and Figure 6-16).

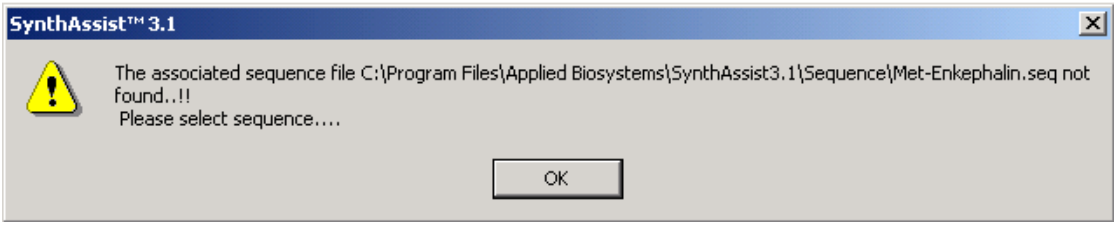


Figure 6-15 Associated Sequence File Was Not Found in the Expected Path

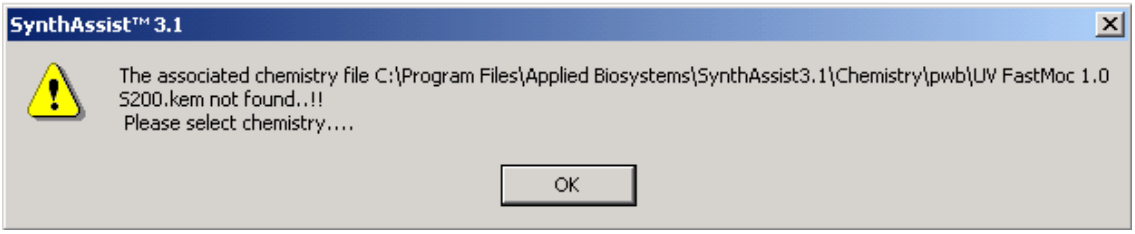


Figure 6-16 Associated Chemistry File Was Not Found in the Expected Path

SynthAssist Software 3.1 opens the File Open dialog box for sequence and chemistry files, respectively, allowing you to select the appropriate files. After associating the files with the Run, save the run by overwriting the existing Run file. This ensures that the next time the Run file is opened, it correctly shows the old values saved in the Run file.

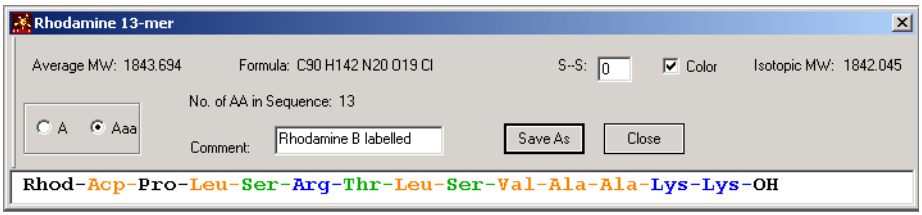
Note: If any change is made to the associated Chemistry and/or Run file(s), then the Calculation view is initialized. You need to enter the appropriate values in the Calculation view, then save it.

Sequence Mismatch

If the sequence in a Run file contains residues that are not defined in the current Dictionary, a Mismatched Sequence window opens (see Figure 6-17). Click **OK** to open the Run with a deleted sequence or click **Cancel**.

If the sequence contains a C-terminus or N-terminus that is not defined in the current Dictionary, an error message will open. Click **OK** to open the Run with substituted termini ("H" at the N-terminus or "OH" at the C-terminus) or click **Cancel**.

For example, a sequence with Rhodamine B at the N-terminus is being opened on a PC whose SynthAssist Dictionary does not contain a definition for the dye.



The following error message appears.

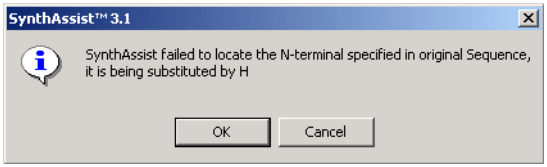


Figure 6-17 Missing N-Terminal Error Message

Creating a Custom Chemistry File

7

This chapter covers:

Overview

New Predefined Chemistry Files

SynthAssist® Software Version 3.1 provides 21 predefined chemistry files in the installation directory:
Applied Biosystems\SynthAssist 3.1\Chemistry. Nineteen of the files are the same as those provided with SynthAssist 2.0 software and two files are new. The new files are:

FT 1–18 Alternate – Contains the same test modules as the standard Flow Tests 1–18, except that the alternate tests sound an alarm and set an interrupt in the modules that require a volume reading at the metering vessel.

FT 19–23 Alternate – Contains a new test in module "c" that pressurizes the MV/RV and checks solvent flow through the activator.

By default, the installed chemistry files are locked or read-only. A lock icon is displayed on the chemistry screen. The contents of the file can be viewed but cannot be modified. An unlocked chemistry file can be created via Save As, providing a new name, and selecting "No" to the question "Lock Chemistry File?" In an unlocked chemistry file the cycles, modules, functions, or reagent names can be changed as necessary.

Note: After creating a custom chemistry, the file can be converted to read-only status via Save As, providing a new name, and selecting "Yes" to the question "Lock Chemistry File?"

Chemistry Software

In SynthAssist software a Chemistry file *e.g.,* FastMoc0.25 mmol, consists of a series of synthesis cycles. During each cycle, one amino acid is added to the peptide-resin by using a series of modules specified by the user. Each module specifies a series of steps or functions that represent reagent deliveries or various mechanical actions, *e.g.,* venting, draining, or mixing.

The organization and hierarchy of the chemistry software is shown in Figure 7-1.

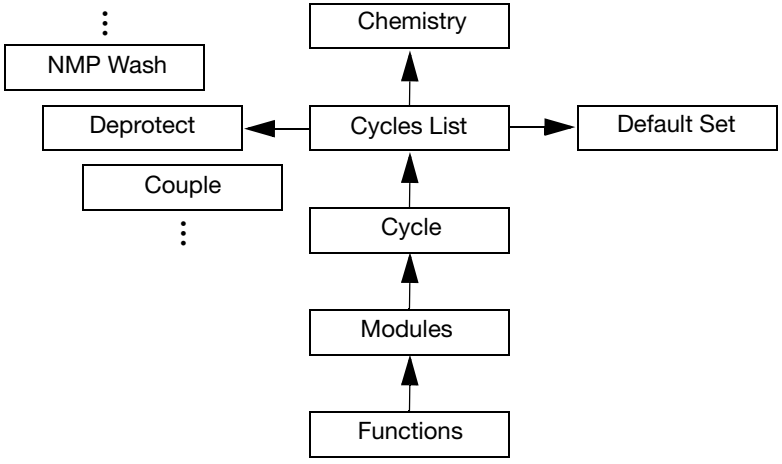


Figure 7-1 Hierarchy of the Chemistry Software

The remainder of this chapter describes how to change reagent names, functions, cycles, modules, and the default set.

Note: You can access and change default sets, cycles, functions, or bottles in any order; you do not need to follow the sequence presented here.

Renaming a Chemistry File

IMPORTANT! Before you can modify a Chemistry file, you must unlock it.

1. Open the Chemistry screen by selecting **File** > **Open**. You can also open the Chemistry screen from the Run screen (click the Chemistry file icon) or from the Lab screen.

   The Chemistry screen opens (Figure 7-2).

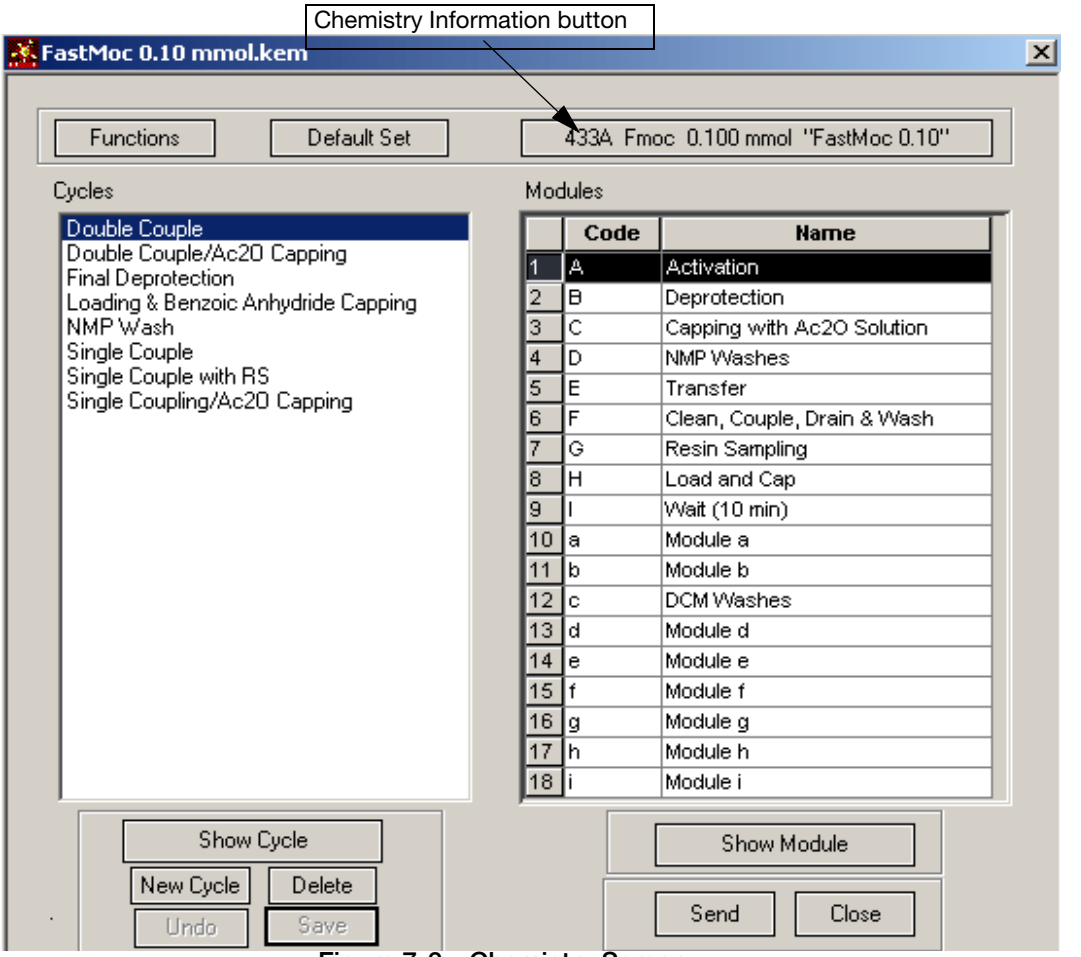


Figure 7-2 Chemistry Screen

Changing the Chemistry Information

To update synthesizer and chemistry parameters:

1. In the Chemistry screen, click the **Chemistry Information** button (Figure 7-2).

   The Chemistry Information dialog box opens (Figure 7-3).

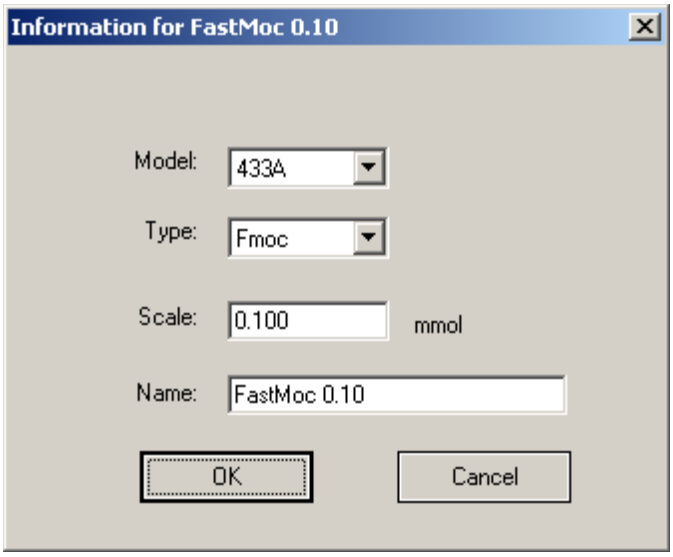


Figure 7-3 Chemistry Information Dialog Box

2. In the Model box, select **433A**.
3. In the Type box, select **Fmoc** or **Boc**.
4. Type in the scale of the chemistry.
5. Type in the name of the chemistry up to 15 characters.
6. Click **OK**.

Changing the Selected Functions

To assess the current functions:

1. In the Chemistry screen, click **Functions**.

   The functions screen opens (Figure 7-4).

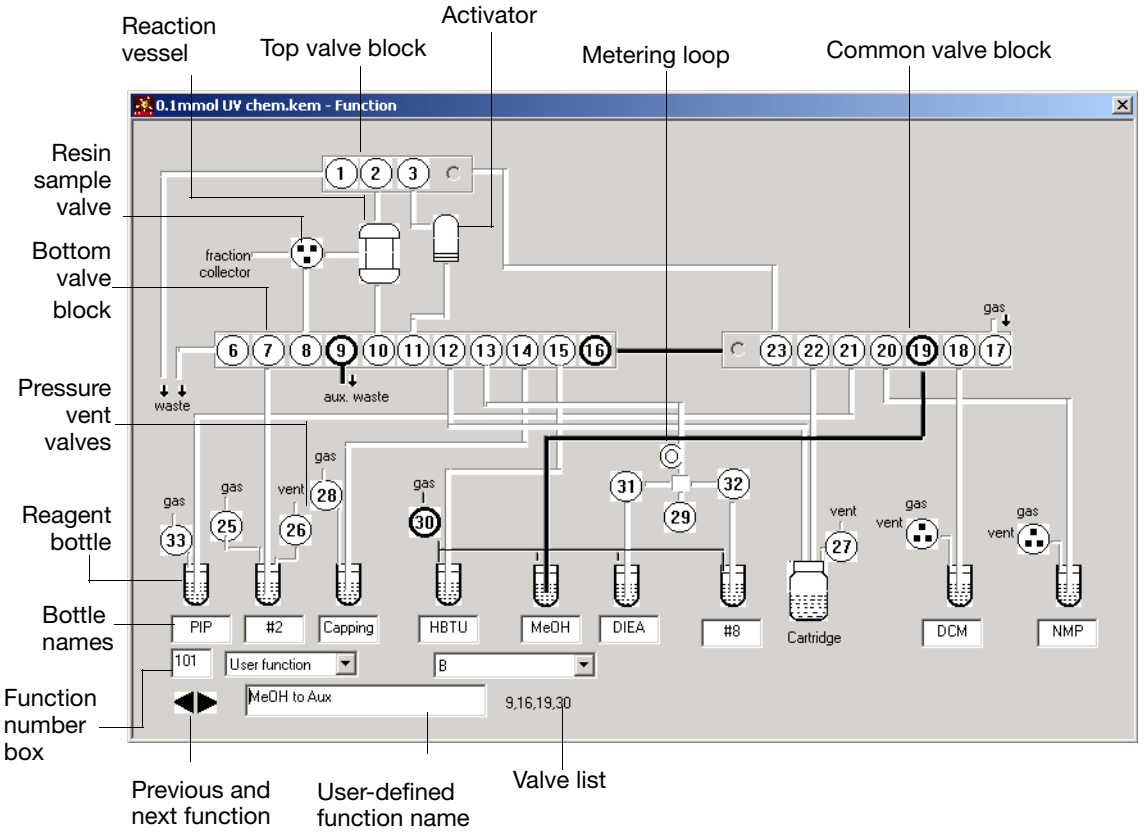


Figure 7-4 Functions Screen

2. Click the **Functions** list box to view the list of functions (Figure 7-5).
3. Use the left and right arrow keys next to the list box to go to a different function.

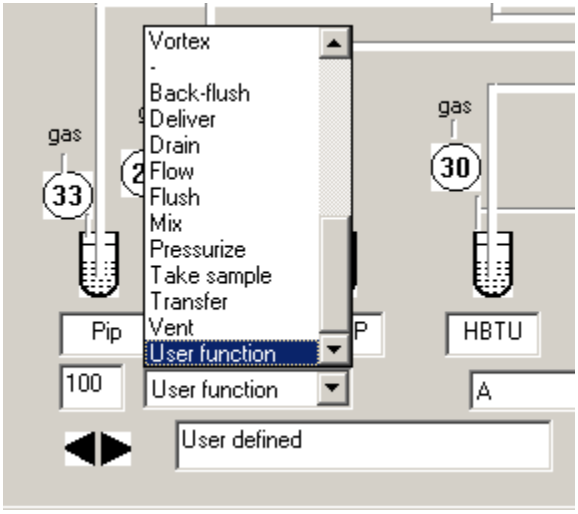


Figure 7-5 The Function List Box

Accessing a Standard Function

Method A

In the Functions screen, click the Functions list box to access standard functions (Figure 7-5).

Method B

If you know the number of the standard function you want, type the number in the Function Number box in the Functions screen, then press **Tab**.

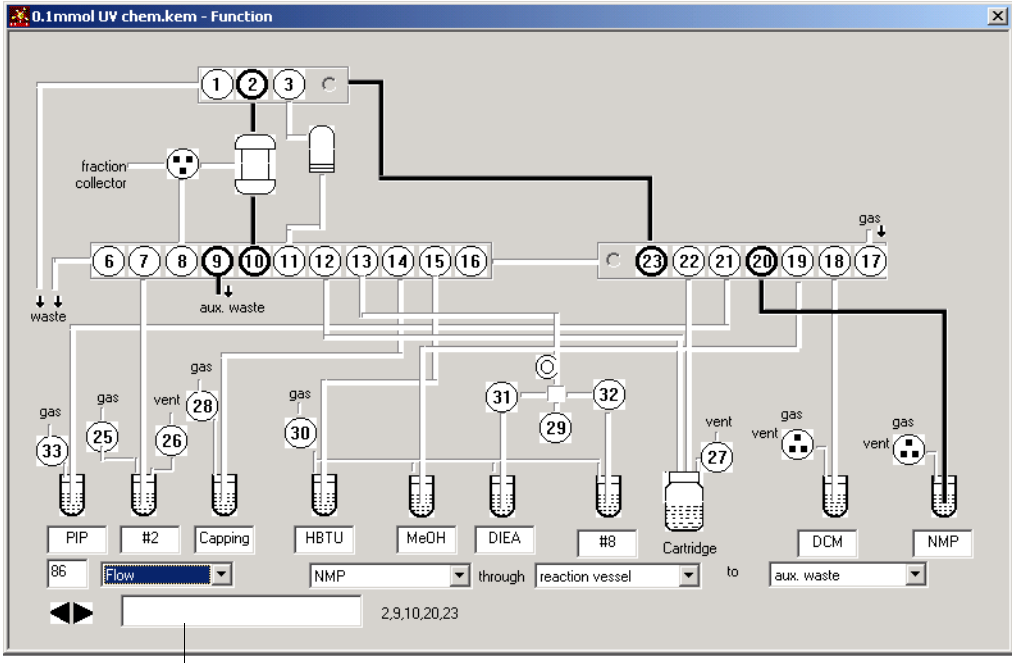


Figure 7-6 Function 86: Flow NMP Through Reaction Vessel to Auxiliary Waste. Open Valves 2, 9, 10, 20, 23

Creating a User-Defined Function

SynthAssist software has 142 predefined (standard) functions and 10 functions which can be user-defined.

To create a user-defined function:

1. In the Function Number box in the Functions screen, type in a function number from 100 to 109, then click **Tab**, or select **User functions** from the Functions list box (Figure 7-5).
2. Edit the valves.
3. Select or deselect a valve to enable or disable the valve.
4. Select or deselect a bottle or vessel to enable or disable the valve that controls the bottle or vessel.

   Note: Six activated valves is the maximum allowed.

5. Type in the name of the new user function in the user-defined Function Name box (Figure 7-6). The user-defined function is created.

Changing the Selected Modules

To change modules:

1. In the Chemistry screen (Figure 7-7), select the module number corresponding to the module you want to change (for example, Activation), then click **Show Module**.

   The module screen opens (Figure 7-8)

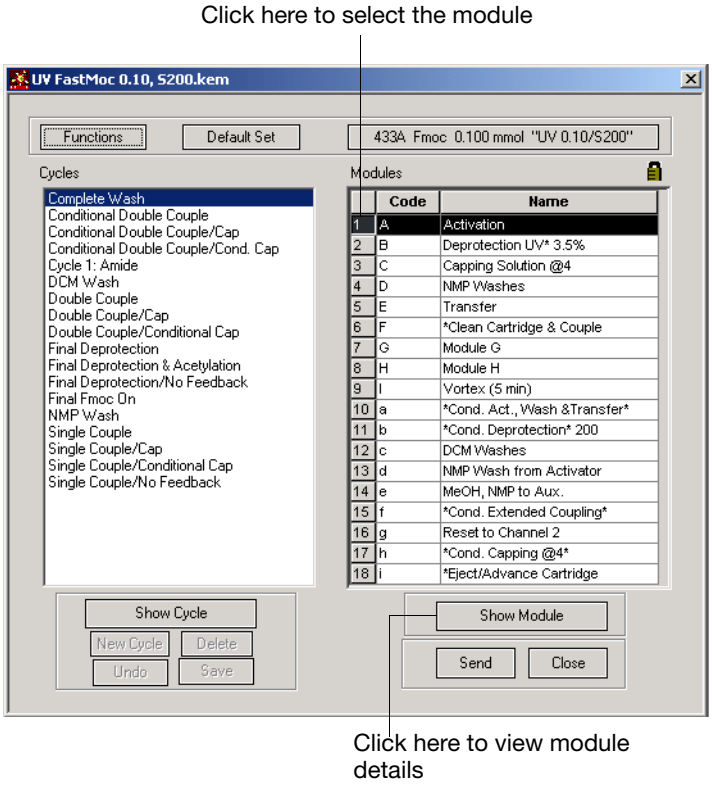
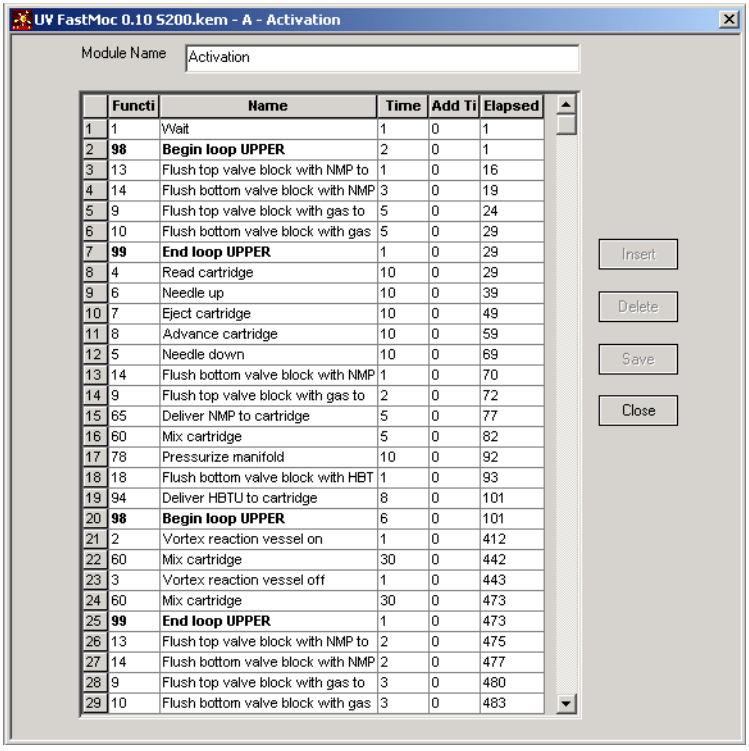


Figure 7-7 Chemistry Screen

Note: A total of 18 modules are available (a through i and A through I). You cannot delete them, but you can change their contents. You cannot add more modules.



	Functi	Name	Time	Add Ti	Elapsed
1	1	Wait	1	0	1
2	**98**	**Begin loop UPPER**	2	0	1
3	13	Flush top valve block with NMP to	1	0	16
4	14	Flush bottom valve block with NMP	3	0	19
5	9	Flush top valve block with gas to	5	0	24
6	10	Flush bottom valve block with gas	5	0	29
7	**99**	**End loop UPPER**	1	0	29
8	4	Read cartridge	10	0	29
9	6	Needle up	10	0	39
10	7	Eject cartridge	10	0	49
11	8	Advance cartridge	10	0	59
12	5	Needle down	10	0	69
13	14	Flush bottom valve block with NMP	1	0	70
14	9	Flush top valve block with gas to	2	0	72
15	65	Deliver NMP to cartridge	5	0	77
16	60	Mix cartridge	5	0	82
17	78	Pressurize manifold	10	0	92
18	18	Flush bottom valve block with HBT	1	0	93
19	94	Deliver HBTU to cartridge	8	0	101
20	**98**	**Begin loop UPPER**	6	0	101
21	2	Vortex reaction vessel on	1	0	412
22	60	Mix cartridge	30	0	442
23	3	Vortex reaction vessel off	1	0	443
24	60	Mix cartridge	30	0	473
25	**99**	**End loop UPPER**	1	0	473
26	13	Flush top valve block with NMP to	2	0	475
27	14	Flush bottom valve block with NMP	2	0	477
28	9	Flush top valve block with gas to	3	0	480
29	10	Flush bottom valve block with gas	3	0	483

Figure 7-8 Module Screen

There are two ways to change the current function in the Module screen:

Method A:

1. Select the name of the function you want to change.

   The Function dialog box opens (Figure 7-9).

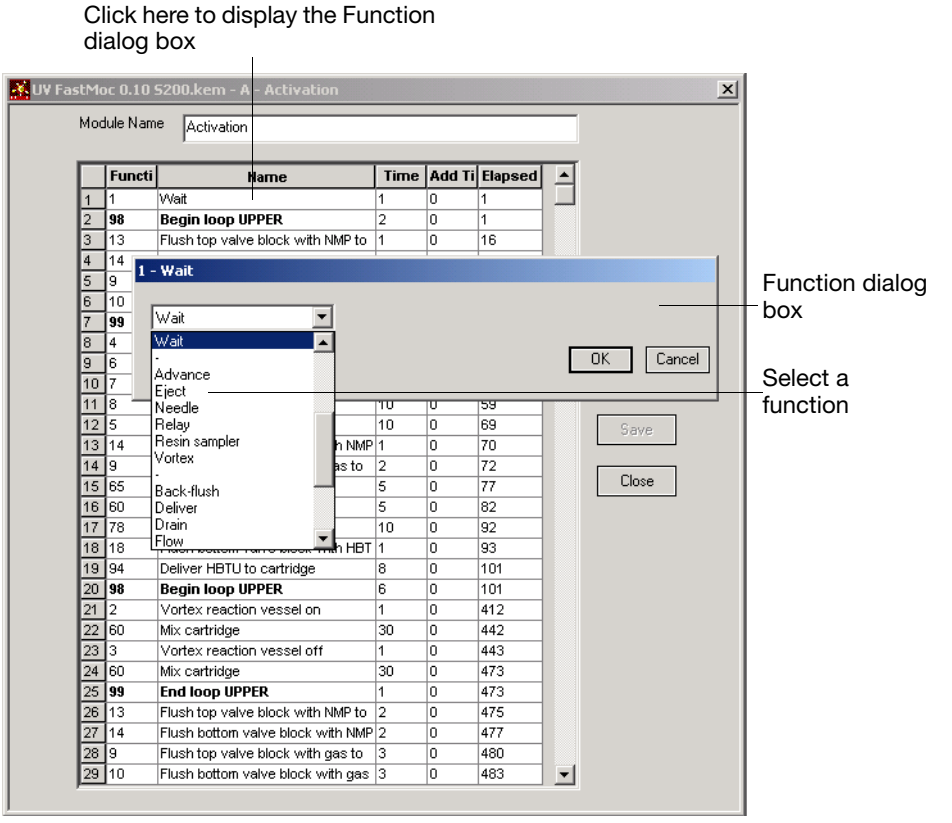


Figure 7-9 Function Dialog Box

2. Select a different function, then click **OK**.

Method B:

Via the Functions screen type the number of the function in the Function number box (Figure 7-4), press **Tab**, then click **OK**.

Changing the Duration of a Step

To change the duration of a step:

1. In the Time column, enter the time (in seconds) for the step to operate. In the monitoring functions Time may be a value other than seconds.

   Use the arrow and numeric keys to select and change the entries in the screen.

   Note: Time for the loop functions represents the number of loops. Time for the monitoring functions, *e.g.,* 134 or 145, represents a deprotection value.

2. Select **Synthesizer** > **Send** to upload the module to the synthesizer.
3. Close the Module screen by clicking **Close**.

Renaming a Module

To rename a module in the Module screen:

Type a new name in the Module Name field, then press **Tab**.

Inserting a Step

To insert a step in the Module screen:

1. Highlight the row above which you wish to insert a step.
2. Click the **Insert** button, or select **Edit** > **Insert**.

   A blank line is inserted below the row you selected.

   IMPORTANT! A module may contain a maximum of 99 steps.

Deleting a Step

To delete a step in the Module screen:

1. Highlight the row you want to delete.
2. Click **Delete,** or select **Edit** > **Delete**.

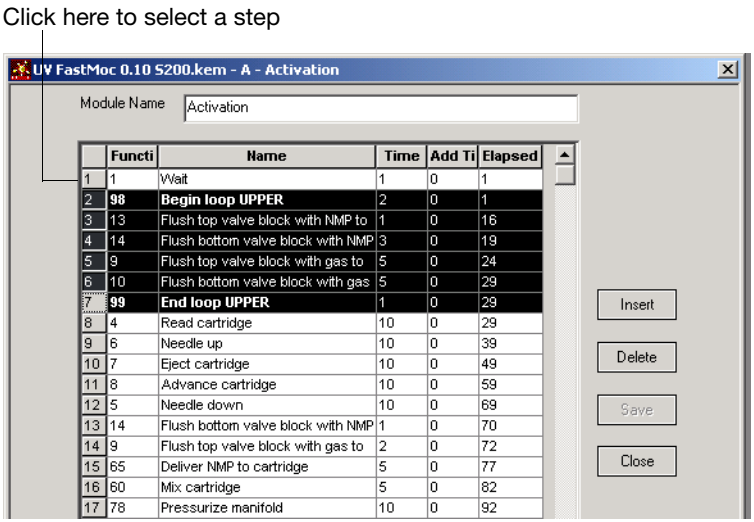
Using Copy/Paste in a Module

To use copy and paste in a module in the Module screen:

1. Open the Module screen of the Chemistry file from which you want to copy steps.
2. Click the number of the cell corresponding to the step to copy (Figure 7-10).

   Use Ctrl-click to select multiple steps or drag the mouse.

3. Press **Ctrl+C** to copy the steps.



	Functi	Name	Time	Add Ti	Elapsed
1	1	Wait	1	0	1
2	**98**	**Begin loop UPPER**	2	0	1
3	13	Flush top valve block with NMP to	1	0	16
4	14	Flush bottom valve block with NMP	3	0	19
5	9	Flush top valve block with gas to	5	0	24
6	10	Flush bottom valve block with gas	5	0	29
7	**99**	**End loop UPPER**	1	0	29
8	4	Read cartridge	10	0	29
9	6	Needle up	10	0	39
10	7	Eject cartridge	10	0	49
11	8	Advance cartridge	10	0	59
12	5	Needle down	10	0	69
13	14	Flush bottom valve block with NMP	1	0	70
14	9	Flush top valve block with gas to	2	0	72
15	65	Deliver NMP to cartridge	5	0	77
16	60	Mix cartridge	5	0	82
17	78	Pressurize manifold	10	0	92

Figure 7-10 Selecting a Step

Note: To select all steps, select the first row, then Shift-click the number of the last row.

4. Open the Module screen of the Chemistry file where you wish to paste the steps.
5. Select the step where you want to paste the steps.
6. Press **Ctrl+V** to paste the copied steps.

Changing Cycles

You can change the modules that define a cycle in the Cycle screen.

Selecting a Module

To select a module:

1. In the Chemistry screen, double-click the cycle you want to change.

   The Cycle screen opens (Figure 7-11).

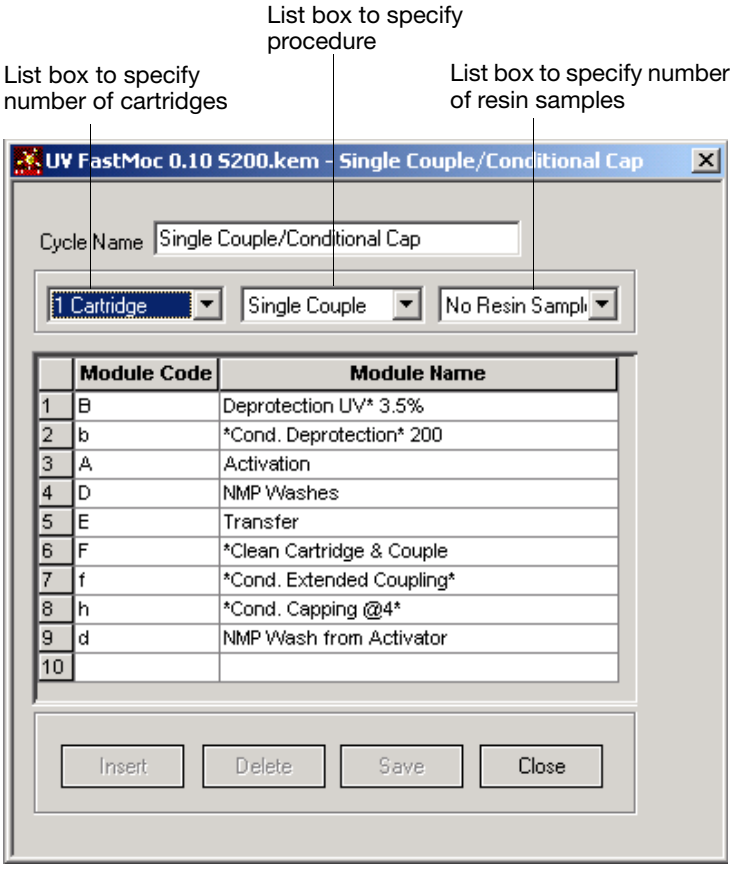


Figure 7-11 Cycle Screen

Changing a Module

Choose one of the following two methods:

Method A:

1. In the **Module Name** column, select the name of the module you want to change.
2. In the drop-down box, select the new module.

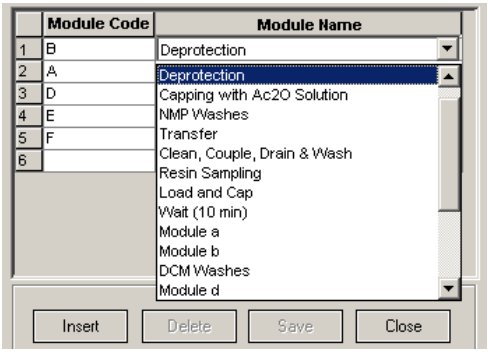


Figure 7-12 Select a Module

Method B:

3. If you know the letter of the new module, type it in the Module Code column, then press **Tab**.
4. Click **Save** to save your changes.

Deleting a Module from the Cycle

To delete a module from the cycle:

1. In the Cycle screen, select the number of the module you want to delete (Figure 7-13).

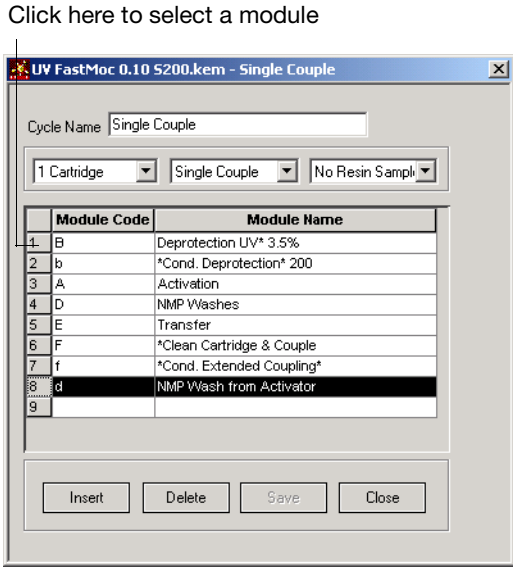


Figure 7-13 Selecting a Module.

2. Click **Delete** or press **Ctrl+K** to delete the module.

Inserting a New Module in the Cycle

To Insert a new module in the cycle:

1. In the cycle screen, highlight the row above which the new module will be inserted.
2. Click **Insert** or press **Ctrl+J** to insert a blank module.
3. Type in the module code for the new module, or select the name of the module in the Module Name column.

   IMPORTANT! A cycle may contain a maximum of 20 modules.

Creating a New Cycle

To create a new cycle:

1. In the Chemistry screen, click **New Cycle** or press **Ctrl+J**.

   A new cycle screen opens with no cycle name (Figure 7-14).
2. Type in a cycle name in the Cycle Name field.

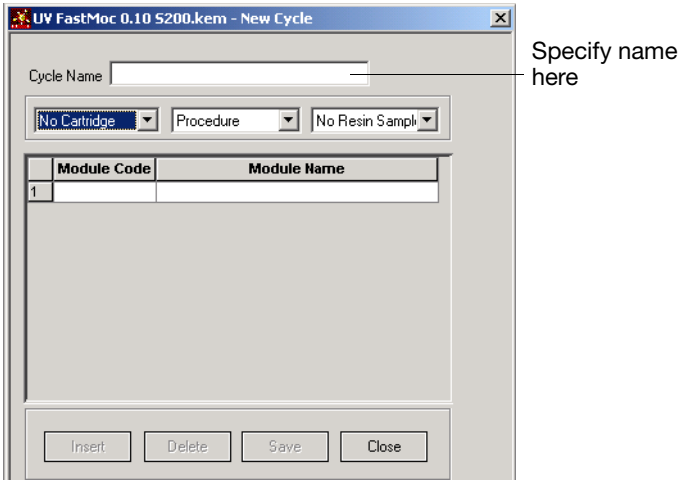


Figure 7-14 Specifying a Cycle Name.

3. Click the **Cartridges** drop-down list box, then select the number of cartridges you want (Figure 7-15).

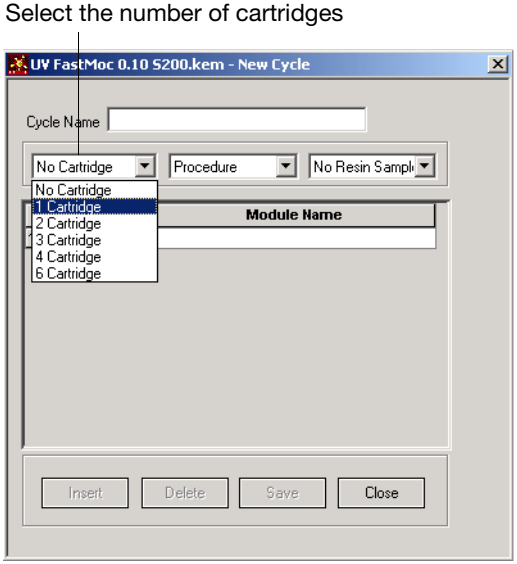


Figure 7-15 Specifying Cartridges

4. Click the **Procedure** drop-down list box, then make the appropriate selection, for example, Single Couple (Figure 7-16).

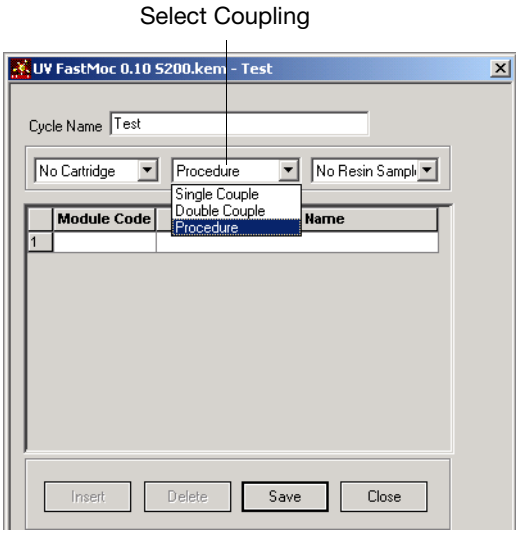


Figure 7-16 Selecting Coupling

IMPORTANT! The standard combinations of cartridge number and coupling are:

- 1 Cartridge, Single Couple;
- 2 Cartridges, Double Couple.

Other cartridge values selected for single or double coupling do not conform to standard usage. SynthAssist software will display a warning message while saving such a cycle.

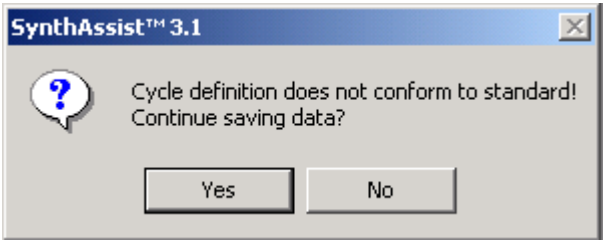


Figure 7-17 Non-conforming Number of Cartridges

Any cartridge value is allowed with Procedure. A minimum of two cartridges is required for Double Couple.

Note: Single coupling at the 1.0 mm scale requires three cartridges. Double coupling at the 1.0 mm scale requires 6 cartridges.

5. Click the **Resin** samples drop-down list box, then make the appropriate selection, for example, No Resin Samples (Figure 7-18).

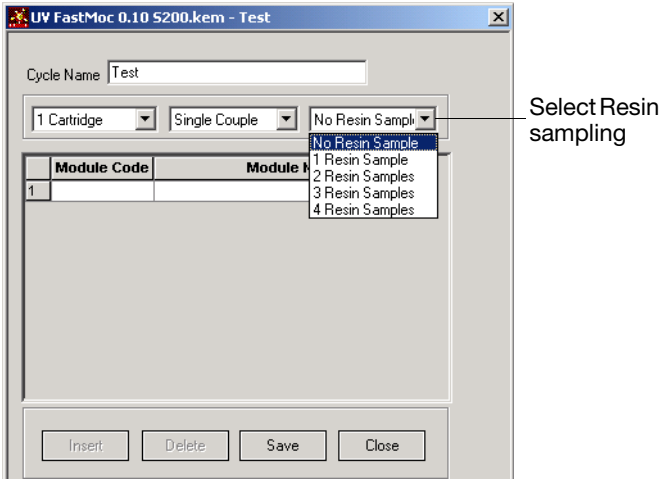


Figure 7-18 Selecting Resin Samples

6. Type in the module letter in the Module Code column, for example, Module A, corresponding to Activation (Figure 7-19).

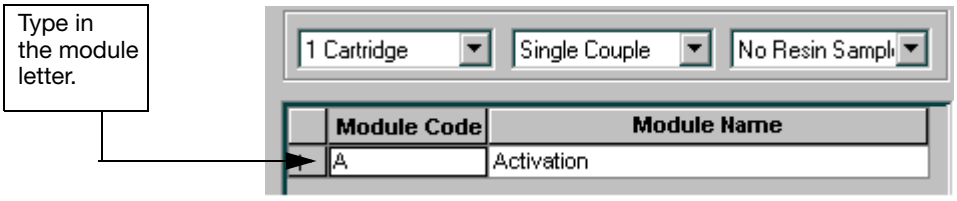


Figure 7-19 Entering a Module

7. Press **Ctrl+S** to save the file.

Deleting a Cycle

To delete a cycle:

1. In the Chemistry screen, select the cycle you want to delete.
2. Select **Edit** > **Delete**.
3. If you make a mistake, select **Edit** > **Undo,** or press **Ctrl+Z**.

Renaming a Cycle

To rename a cycle:

1. In the Chemistry screen, double-click the Cycle you want to rename.
2. in the Cycle Name field, change the cycle name.
3. Press **Ctrl+S** to save the Chemistry.

Creating a New Default Set

A default set consists of a series of preset cycles for each chemistry type (for example, Boc, Fmoc or FastMoc). You can change these preset cycles to create a new default set.

Changing a Default Cycle in a Default Set

To change a default cycle in the default set:

1. In the Chemistry screen, click **Default Set** (Figure 7-20).

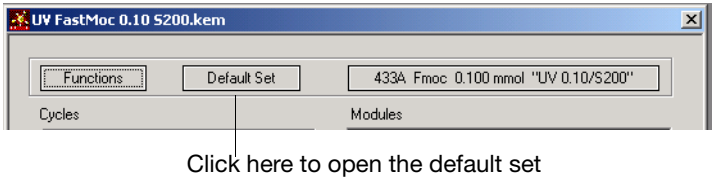


Figure 7-20 Opening the Default Set

The Default Set screen opens (Figure 7-21).

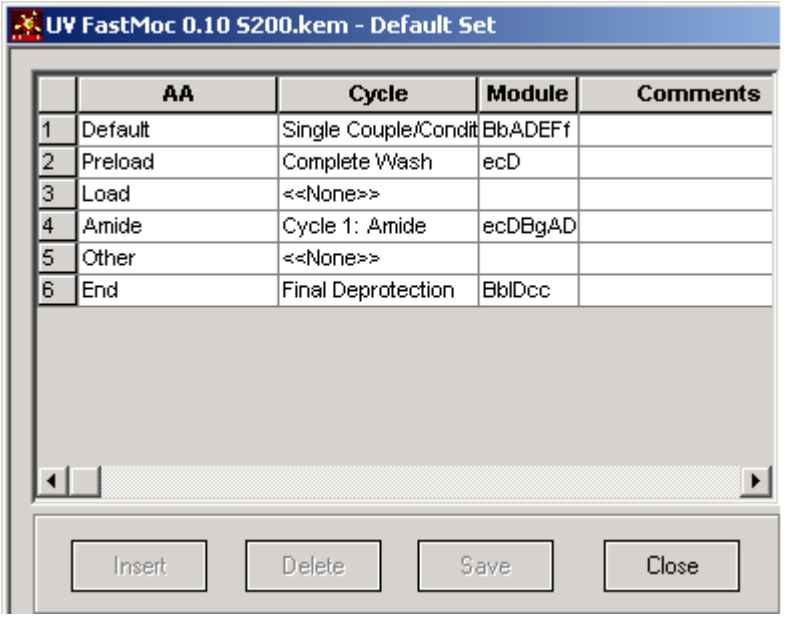


Figure 7-21 The Default Set Screen

2. In the drop-down list of cycles, select the desired cycle (Figure 7-22).

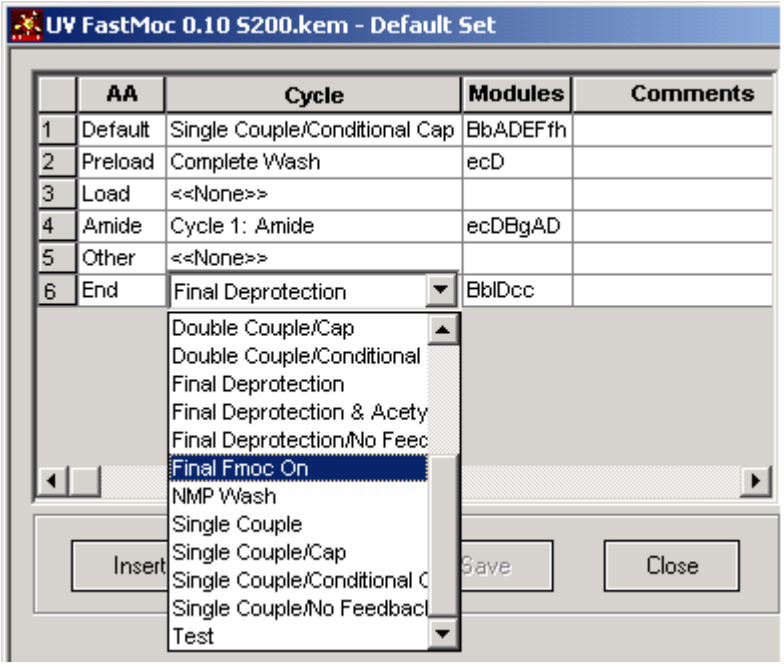


Figure 7-22 Selecting a new Default Cycle

The cycle you select, in this example Final Fmoc On (Figure 7-23), becomes the default End cycle for this Chemistry file.

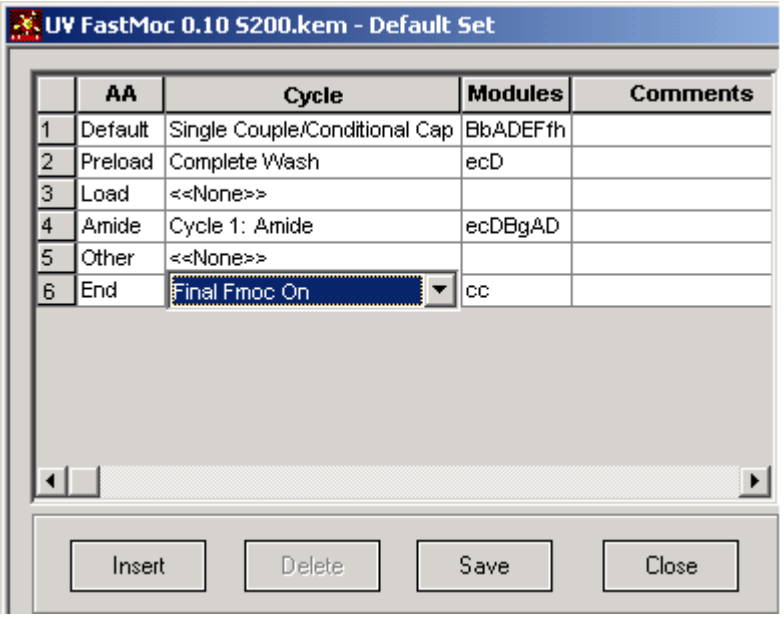


Figure 7-23 A new default cycle for End

IMPORTANT! SynthAssist 3.1 software has two additional AA cells in the default set: Amide and Other.

Inserting Amino Acids in a Default Set

To insert amino acids in a default set:

1. In the Default Set screen, click **End**, then click **Insert**. Repeat, to insert each subsequent amino acid (Figure 7-24).

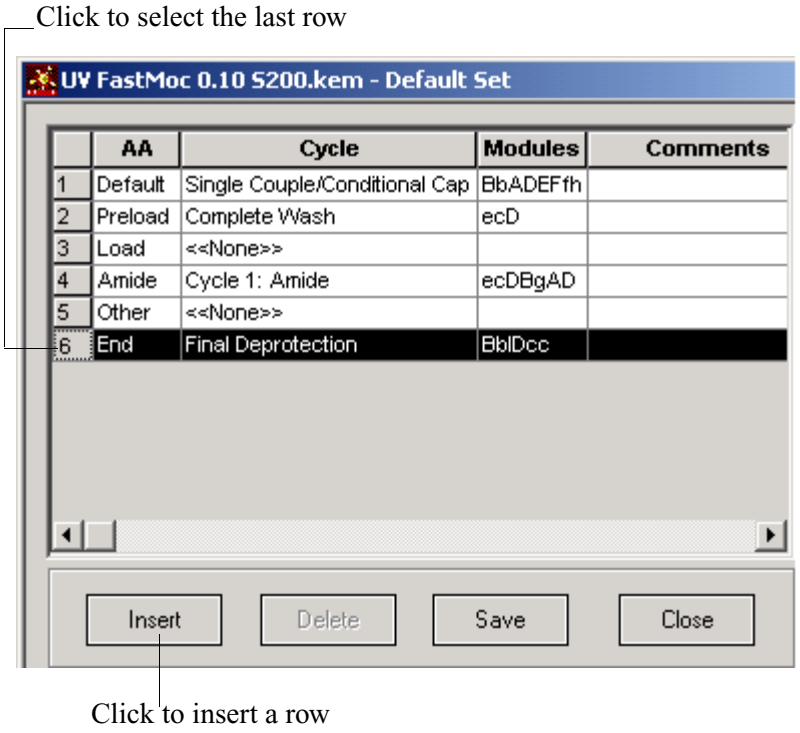


Figure 7-24 Inserting an Amino Acid

2. Click the empty AA field to display the amino acid list (Figure 7-25).

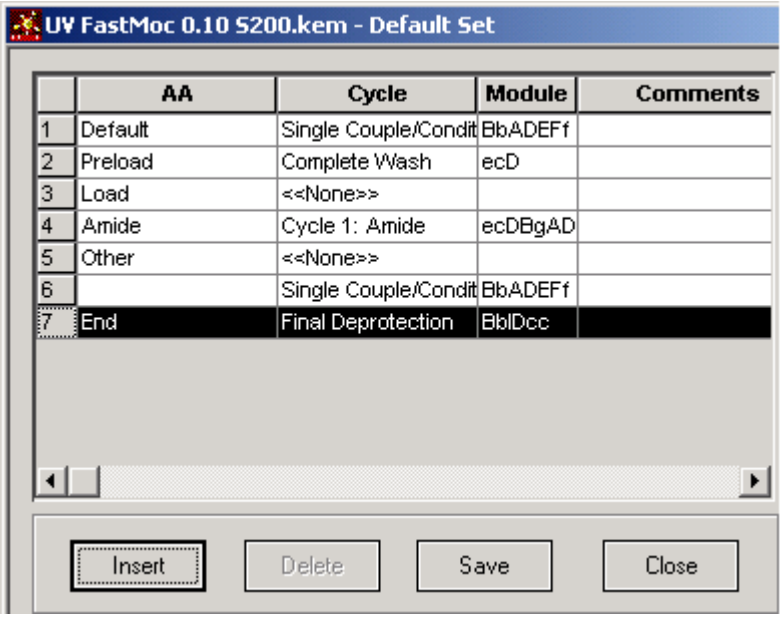


Figure 7-25 Displaying an Amino Acid List

3. Select an amino acid from the drop-down list (Figure 7-26).

   The Default cycle of the chemistry is associated with the new amino acid.

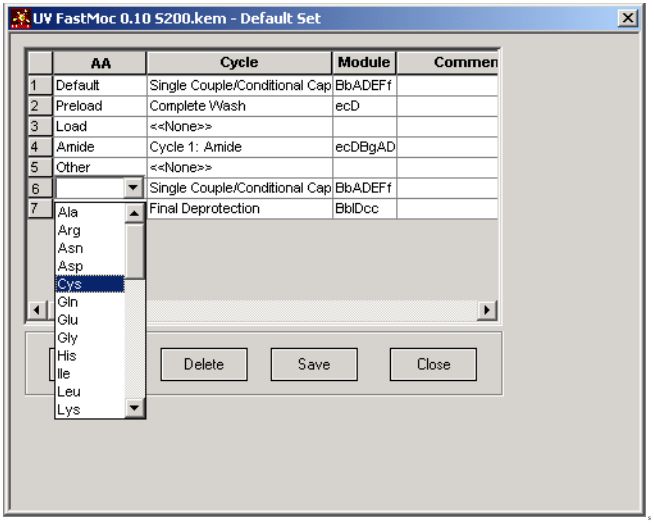


Figure 7-26 Selecting an Amino Acid.

4. To associate a different cycle with the newly inserted amino acid (Asn), click the cell in the cycle column to the right of the amino acid (Figure 7-27).

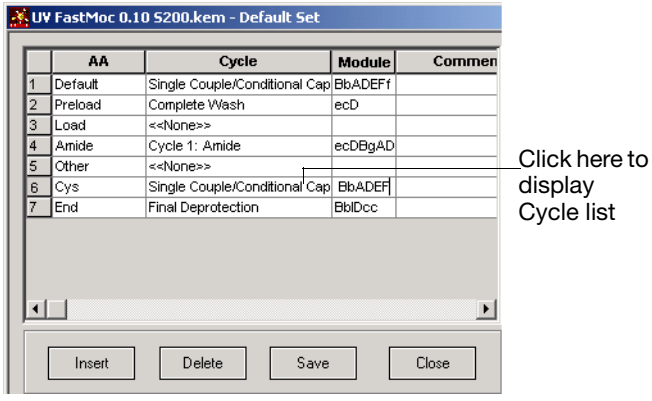


Figure 7-27 Displaying the Cycle List.

5. Select a cycle from the drop-down list (Figure 7-28), then press **Tab**.

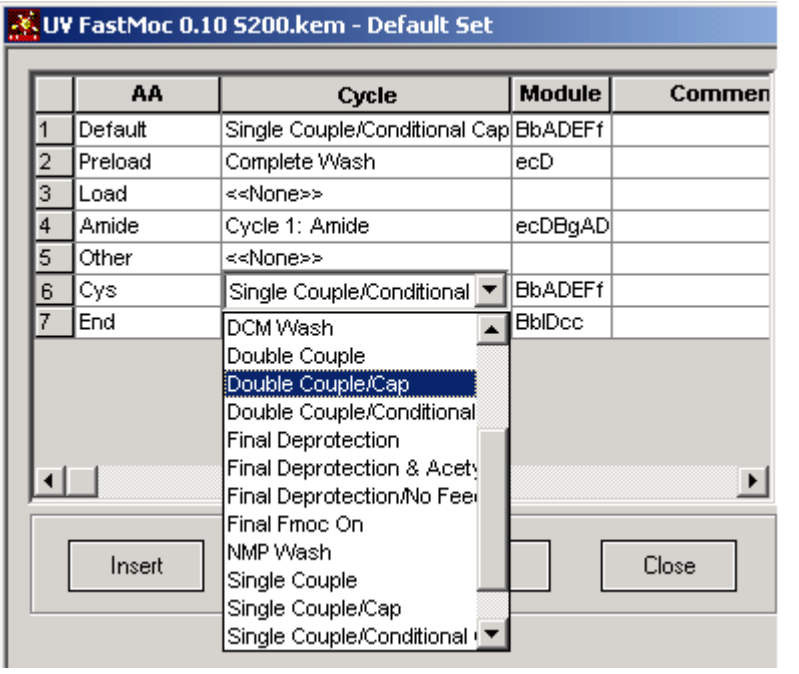


Figure 7-28 Selecting a Cycle for the Newly Inserted Amino Acid

Creating a Non-Default Cycle for an Amino Acid in a Default Set

To create a non-default cycle for an amino acid in the Default Set:

1. Insert the amino acid in the Default Set screen (See "Inserting Amino Acids in a Default Set" on page 7-24).
2. Select **None** as the cycle (Figure 7-29).

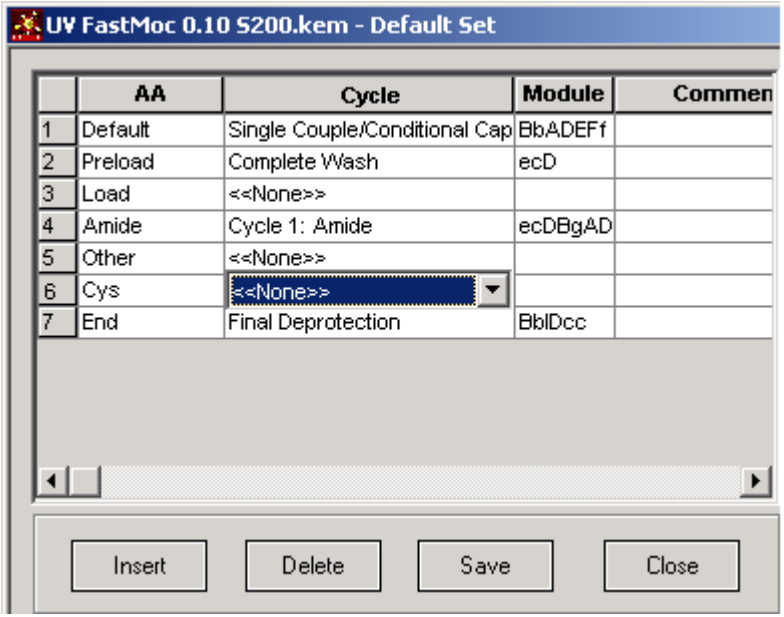


Figure 7-29 Select None as a Default Cycle

You can now enter any combination of modules in the Run screen for Cys. You must choose the module(s) for the amino acid **Cys** in the Run file for all subsequent runs.

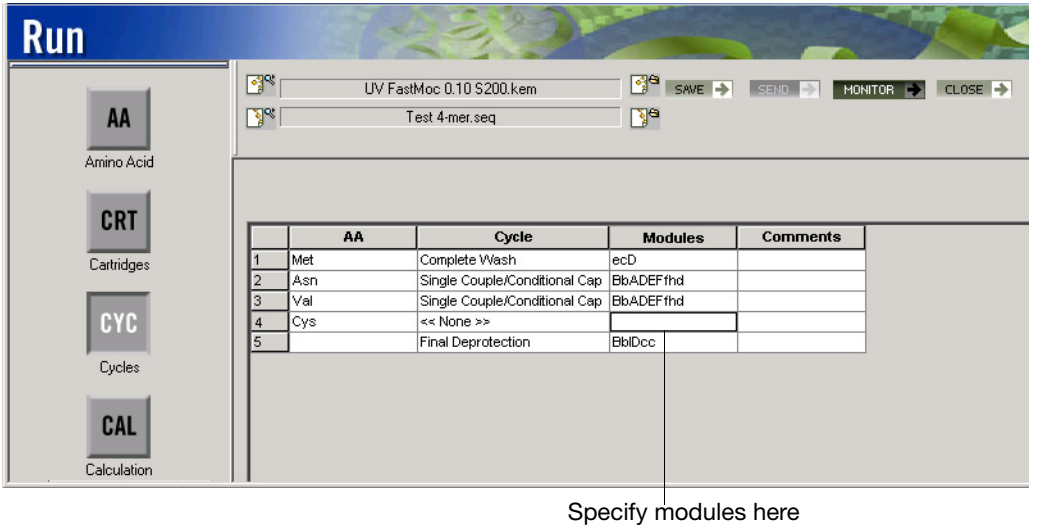


Figure 7-30 Run Screen

Changing a Cycle in the Run Screen

If you change a cycle in the Run screen, the change is associated only with that run, not with the chemistry or with any future runs.

To change a cycle in the Run screen:

1. In the Run screen, click the Cycle cell to the right of Cys.

   A list of cycles is displayed (Figure 7-31).

2. Select a cycle, for example, Double Couple/Conditional Cap, then press **Tab**.

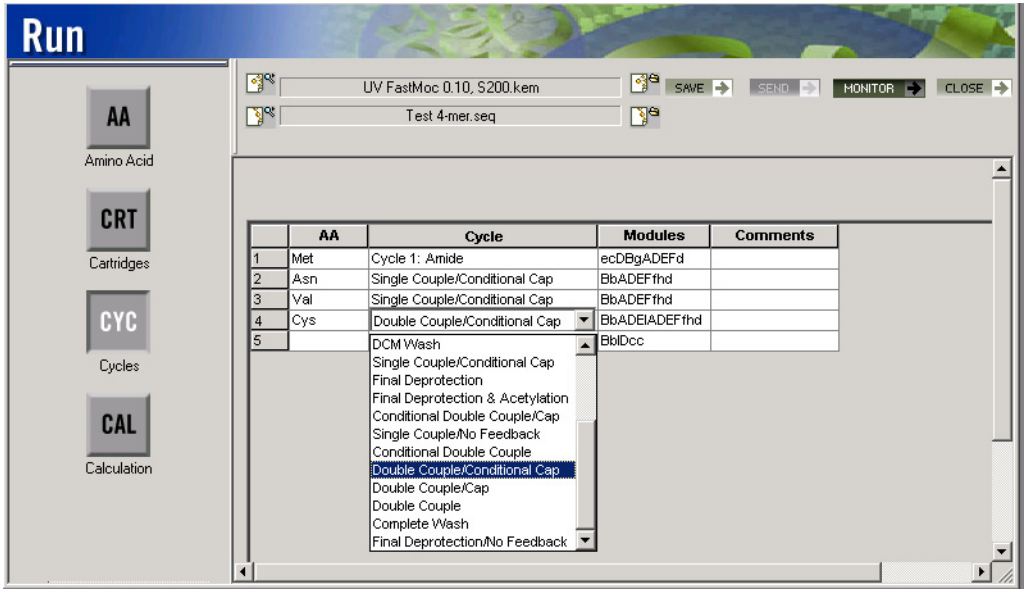


Figure 7-31 Changing a Cycle in the Run Screen

Double Couple/Conditional Cap is now the default cycle for the amino acid Cys for this run only.

Using the Dictionary 8

This chapter covers:

Overview

The Dictionary is a database of all compounds (amino acids, other residues, resins, C-terminals, N-terminals, and protecting groups) that SynthAssist® Software Version 3.1 uses. Applied Biosystems has provided the most commonly used compounds but you can delete or modify these compounds or add new compounds of interest.

Opening the Dictionary

To open the dictionary file:

1. Select **Common > Dictionary** from the main menu.

   The Dictionary screen for amino acids opens (Figure 8-1).

   The default display shows the parameters for the first amino acid in the list on the left. If you select another amino acid, the display shows the parameters for that amino acid (for example, Arg as shown in Figure 8-1).

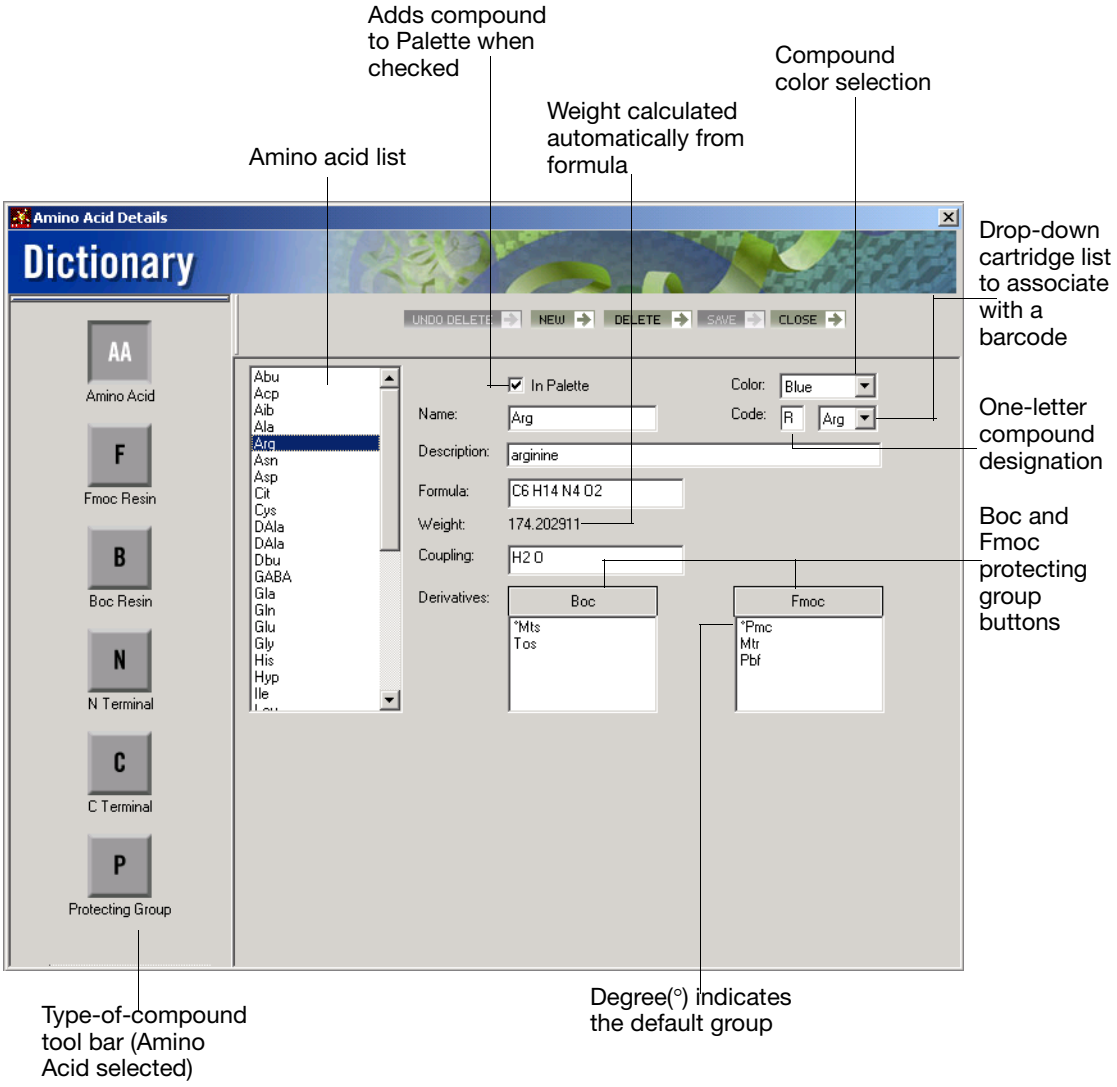


Figure 8-1 Dictionary Screen

Adding a Compound to the Palette

In the Dictionary screen, select **In Palette**. A ☑ indicates the compound is in the palette.

IMPORTANT! If you select a non-alphabetic character (for example, a dash or a space) as the single letter code for an amino acid, then that amino acid is not selectable using the keyboard in the Sequence screen.

Adding Color to a Compound

In the Dictionary screen, select a color in the color drop-down list box. The currently selected compound is displayed in this color in the Sequence screen and in the Run Monitor screen.

Note: Default colors for compounds are color coded:

- Black and orange indicate hydrophobic compounds or unknowns.
- Green and blue indicate basic hydrophilic compounds.
- Red indicates acidic hydrophilic compounds.
- Purple indicates -SH residues.

Changing the Default Derivative of an Amino Acid

To change the default derivative of an amino acid:

1. In the Dictionary screen (Figure 8-1), click **Boc** or **Fmoc**.

   The Add/Remove dialog box opens.

2. Select a protecting group (Pmc) in the Fmoc derivatives list (Figure 8-2).

   The degree symbol (°) to the left of Pmc indicates that Pmc is now the default protecting group.

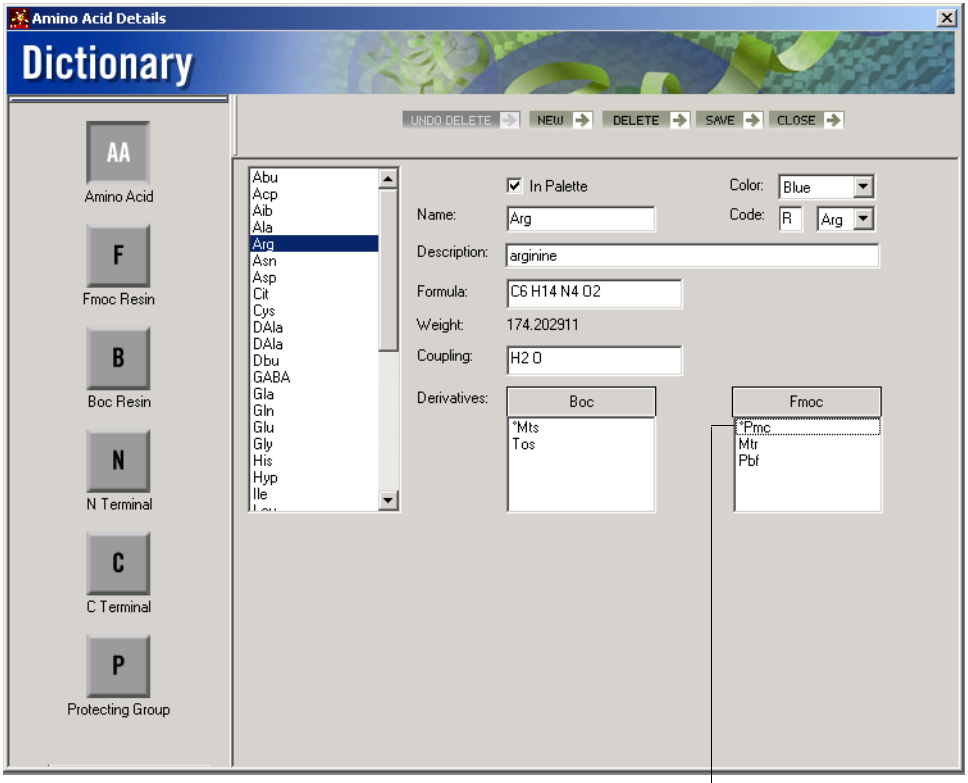


Figure 8-2 Changing the Default Protecting Group

Adding (or Removing) a Protecting Group

To add a protecting group to, or to remove a protecting group from an amino acid:

1. In the Dictionary screen (Figure 8-1), click the **Boc** or **Fmoc** button (Fmoc and Arg are used in this example).

   The Protecting Group Selection dialog box opens (Figure 8-3).

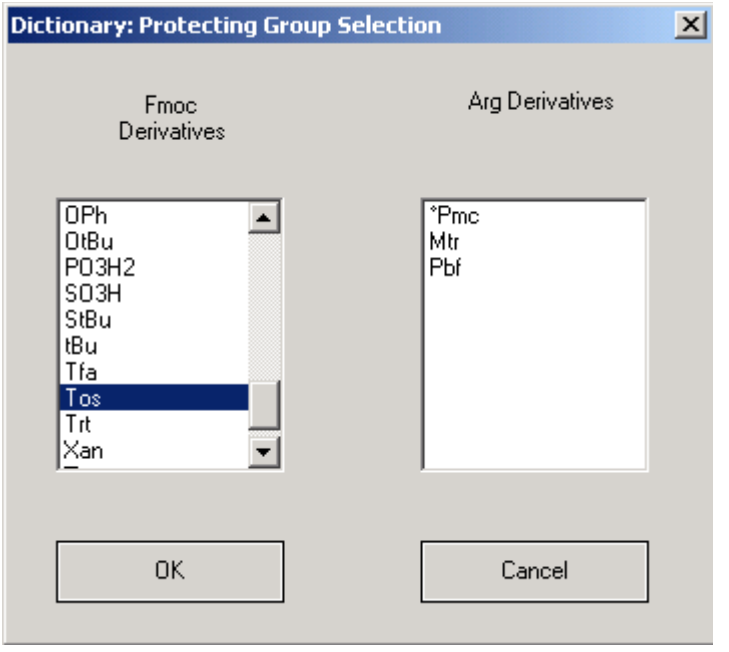


Figure 8-3 Select a new Fmoc Protecting Group

2. To add a protecting group, scroll the Fmoc Derivatives list and double-click the group (Figure 8-3). The new group (Tos) will be added to the Arg derivatives list (Figure 8-4).

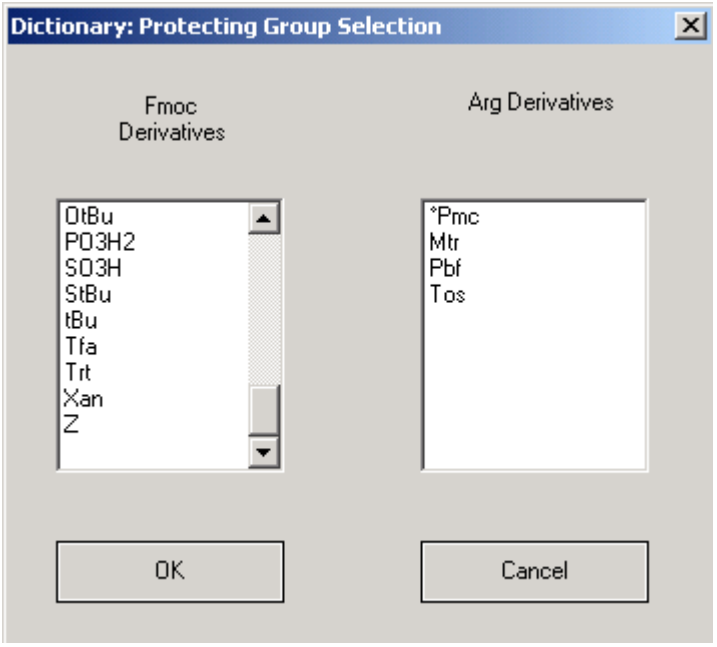


Figure 8-4 The new Protecting Group (Tos)

3. To delete a protecting group, double-click the group in the Arg Derivatives list. Protecting groups can also be dragged between the two lists.
4. Click **Ok**. The addition or deletion will appear on the Amino Acid page of the Dictionary.

Changing Resins

To change resins:

1. Select **Fmoc** or **Bmoc** Resin in the vertical tool bar of the Dictionary screen.

   The display for resins opens (Figure 8-5). Fmoc Resin is used in this example.

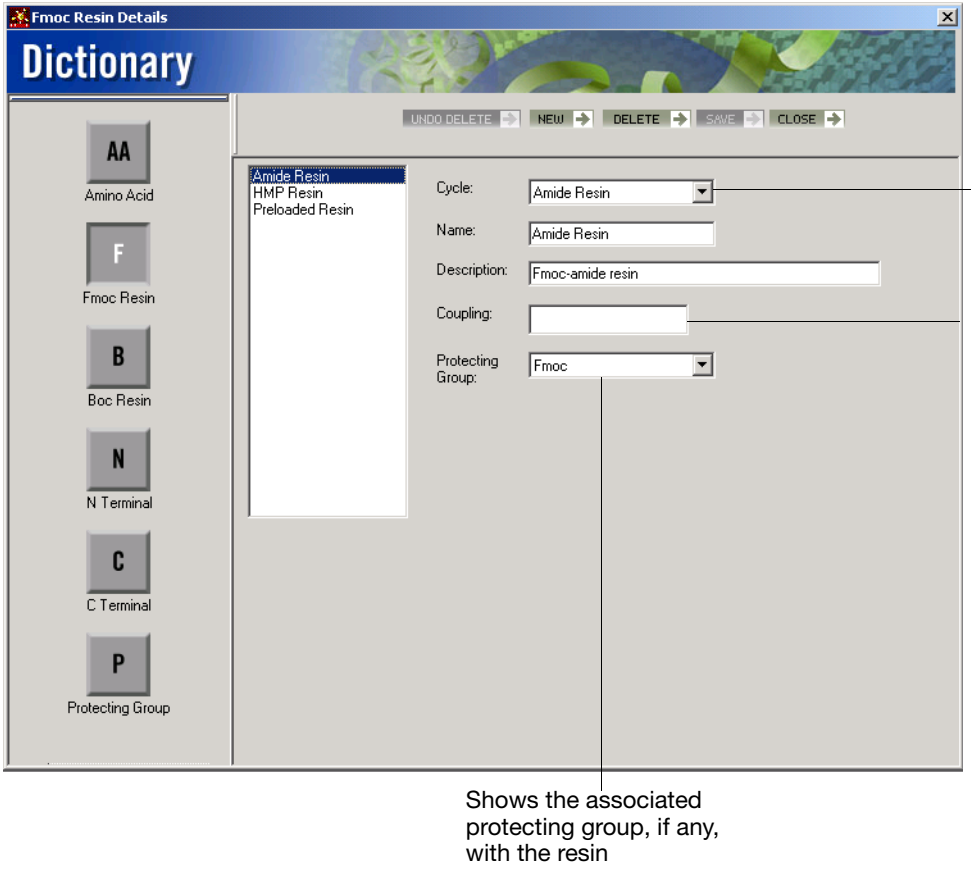


Figure 8-5 Resins Display

2. Type the changes in the appropriate fields, select the appropriate resin field, then press **Tab**.

Adding a Resin

To add a resin:

1. Select **Edit** > **Insert**, or click **New** in the toolbar at the top of the Dictionary screen.
2. If the first amino acid is attached to the resin, select **Preloaded Resin** in the list box.

   Note: SynthAssist 3.1 software has four types of cycles that can be associated with resins.

   - Amide cycle
   - Preloaded
   - Load
   - Other
3. Type in the name, description, coupling, and protecting group, if applicable.

   The Default Protecting group for:
   - All Fmoc resins is Fmoc
   - For all Boc resins is Boc

Adding a Terminal

To add a terminal:

1. Select **C-Terminal** or **N-Terminal** in the vertical tool bar of the Dictionary screen.

   The display for terminals opens (Figure 8-6). C-Terminal is used in this example.

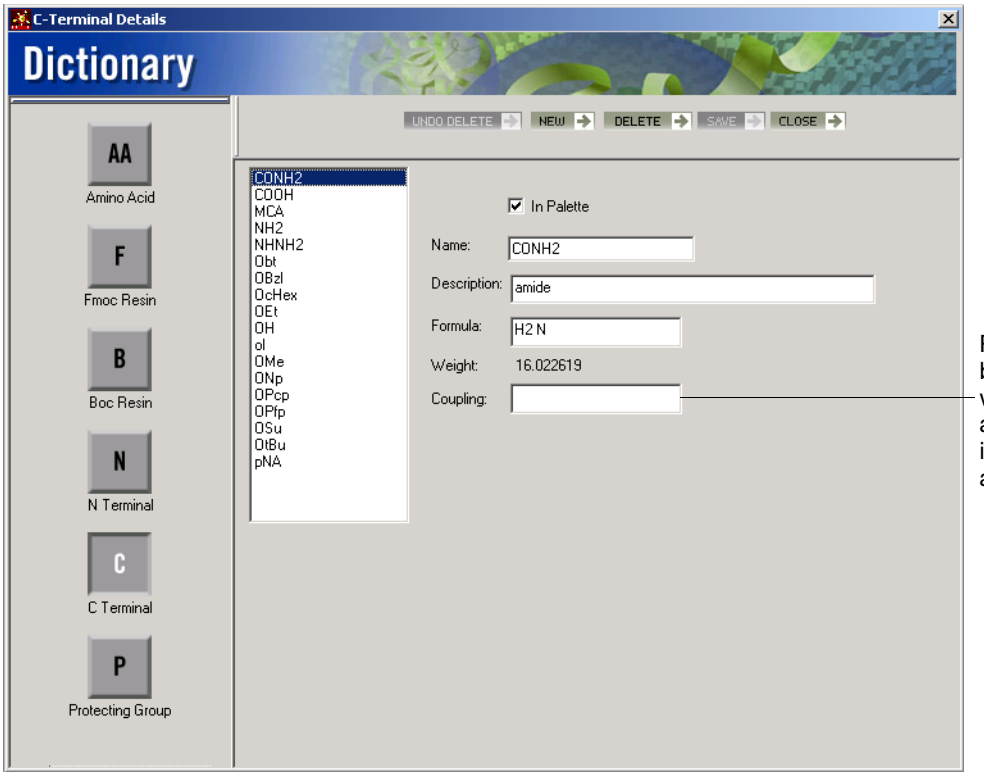


Figure 8-6 C-Terminal Display

2. Select **Edit** > **Insert**.

   If you want the terminal to appear in the palette, select **In Palette**.

3. Type the name, description, formula, and coupling, if applicable, then press **Tab,** or click another field.

   SynthAssist software calculates the molecular weight.

Adding a Protecting Group

To add a protecting group:

1. Select **Protecting Group** in the vertical tool bar of the Dictionary screen.

   The display for protecting group opens (Figure 8-7).

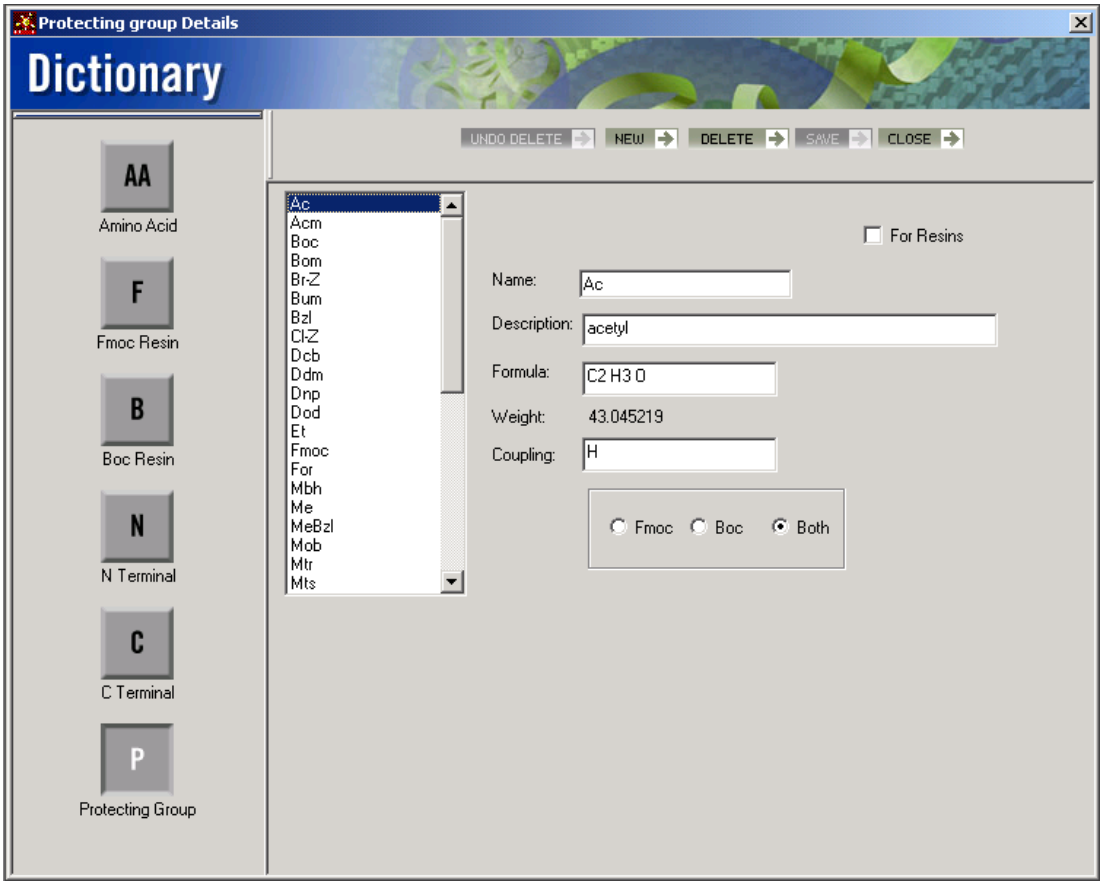


Figure 8-7 Protecting Group Display

2. Select **Edit > Insert**, or click **New** in the top toolbar in the Dictionary screen.

3. Type the name, description, formula and coupling, if applicable, then click **Save** or select **File > Save**.

   SynthAssist software calculates the molecular weight.

   Note: You can specify protecting groups as Boc groups, as Fmoc groups, or as groups common to both Boc and Fmoc chemistry. If a protecting group is specified as Boc, then this group appears only in the Boc list for a particular amino acid or other residue. Similarly, if a protecting group is specified as Fmoc, it appears only in the Fmoc list for a given residue. As a default at installation, all protecting groups are specified as both.

   Note: Among the list of protecting groups, only Boc and Fmoc are selected "For Resin." As such, the Fmoc-weight is subtracted in the final line, H, of the calculations page for an Fmoc-chemistry run. A similar subtraction occurs for the Boc group in a Boc-chemistry run.

SynthAssist® Software Menus

A

This appendix covers:

List of Menus

The SynthAssist software menu bar has nine drop-down menus, almost all of which have commands with corresponding tool bar button commands. The menus are:

- File menu
- Edit menu
- View menu
- Synthesizer menu
- Data Converter menu
- Common menu
- Log menu
- Window menu
- Help menu

File Menu

File

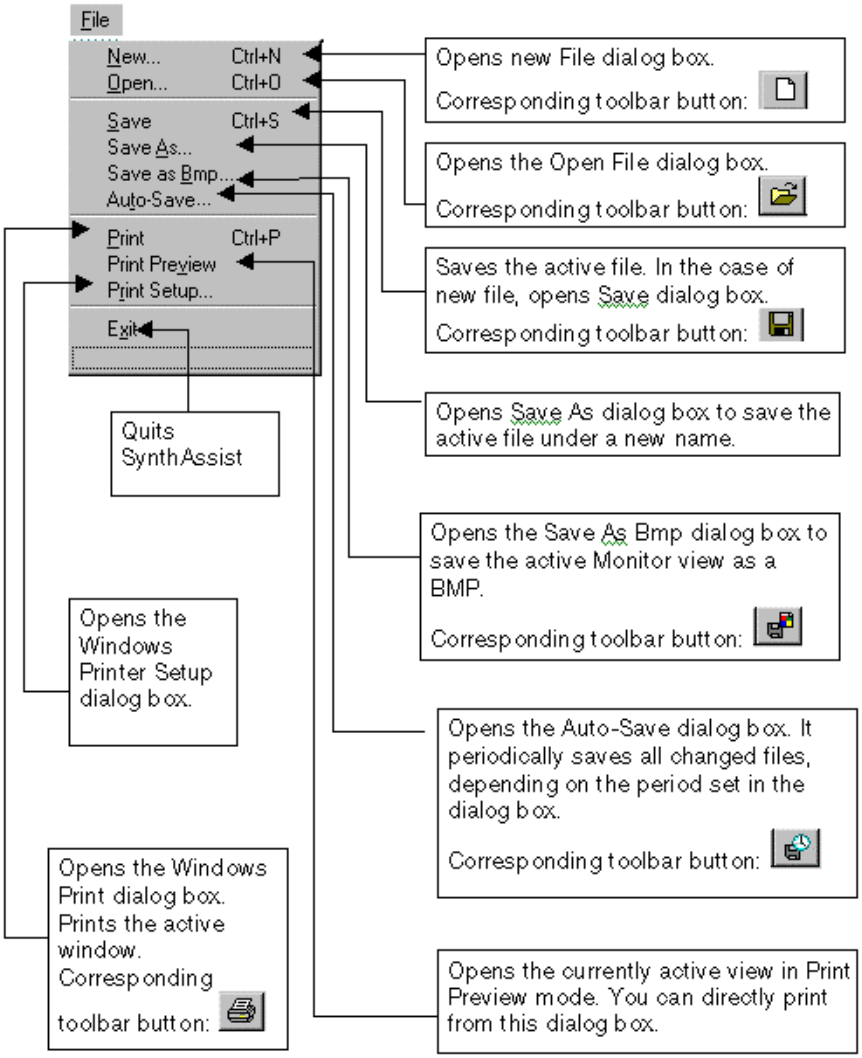


Figure A-1 File Menu Commands

Edit Menu

Edit

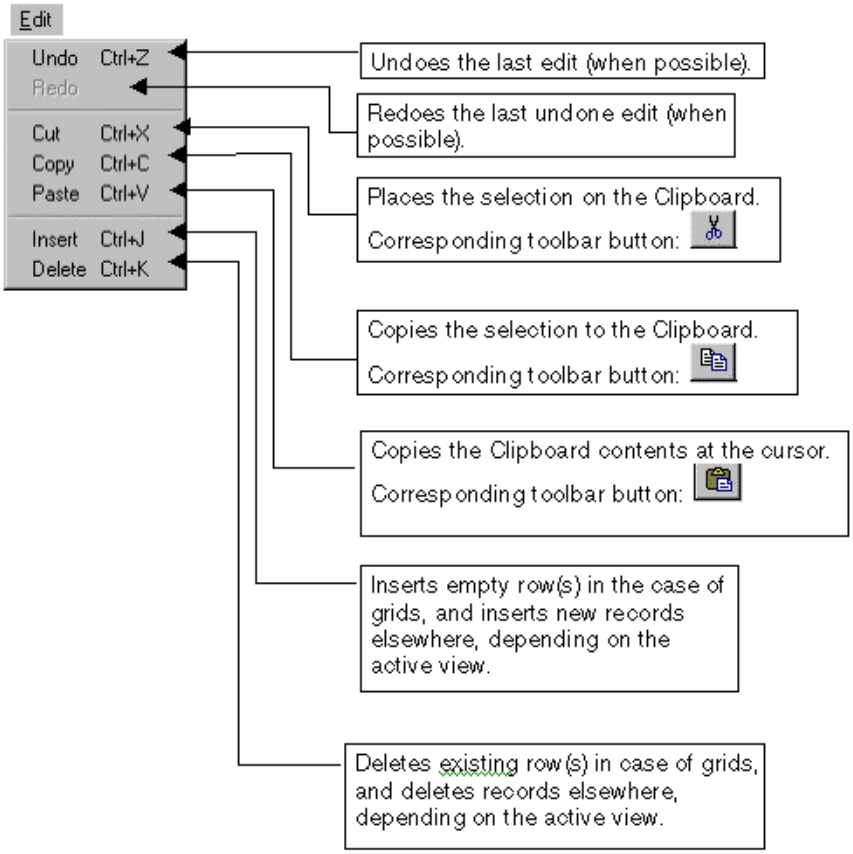


Figure A-2 Edit Menu Commands

View Menu

View

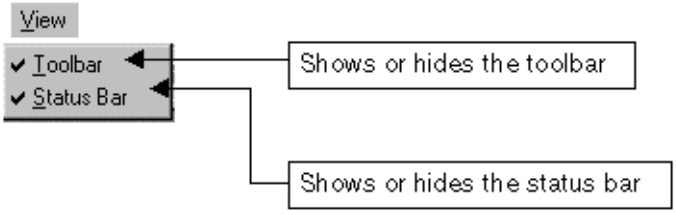


Figure A-3 View Menu Commands

Synthesizer Menu

Synthesizer

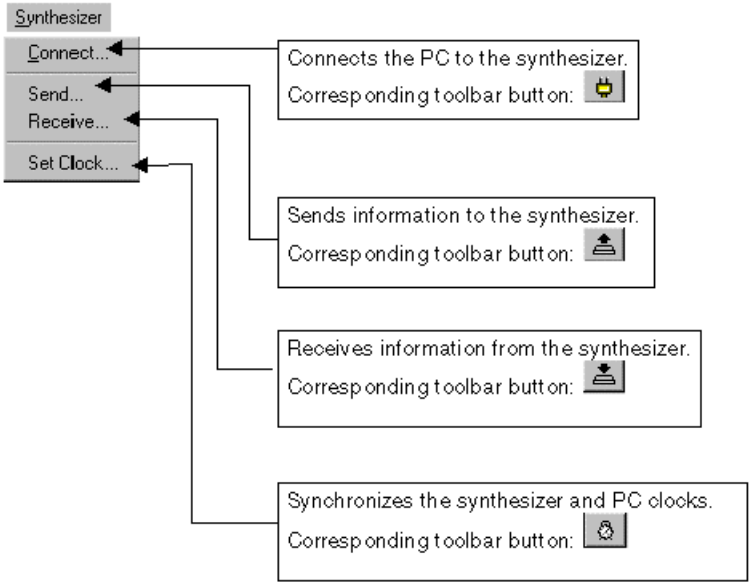


Figure A-4 Synthesizer Menu Commands

Data Converter Menu

Data Converter

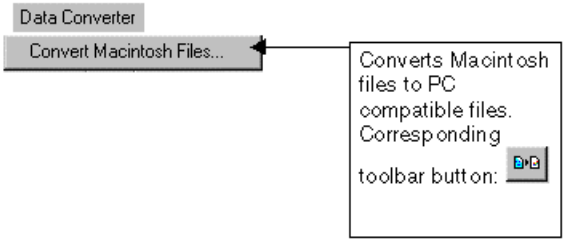


Figure A-5 Data Converter Menu Command

Common Menu

Common

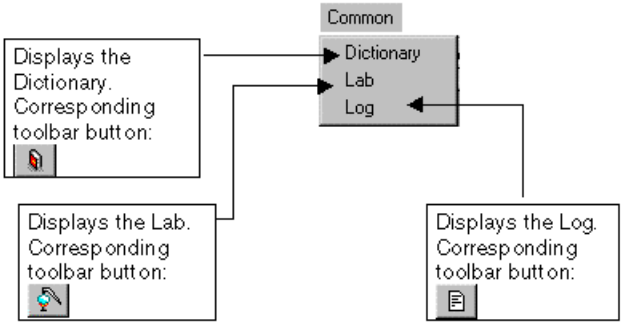


Figure A-6 Common Menu Commands

Log Menu

Log

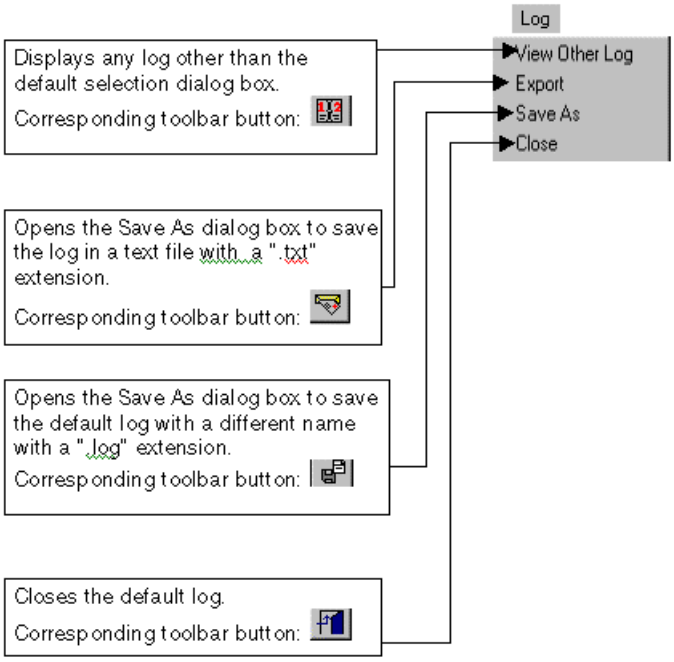


Figure A-7 Log Menu Commands

Window Menu

Window

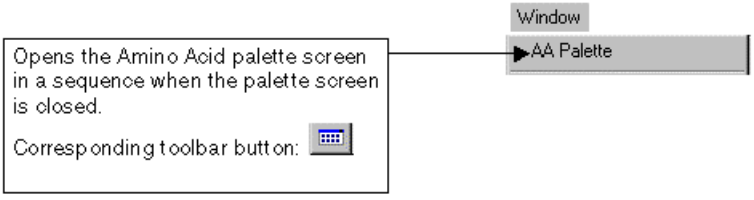


Figure A-8 Window Menu Commands

Help Menu

Help

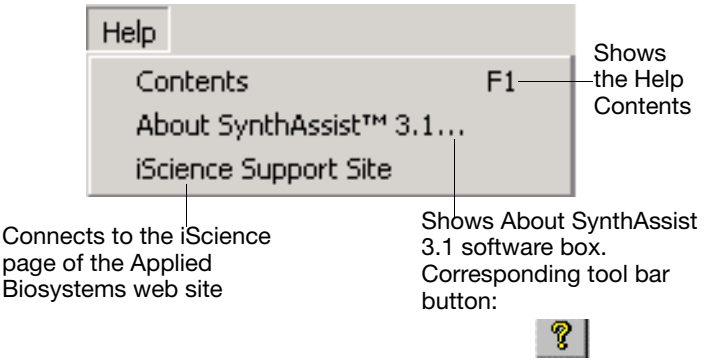


Figure A-9 Help Menu Commands

SynthAssist® Software Screens Overview

B

The following relationships exist among the various SynthAssist software screens:

- From the SynthAssist software menu bar, you can access:
  - File-related dialog boxes and commands
  - Online Help
  - Synthesizer-related dialog boxes
  - The Data Converter dialog box
  - The Dictionary screen
  - The Lab screen
  - The Log screen
  - The Other Log screen
  - The Auto-Save dialog box
  - The Save As Bmp (only for the monitor screen of a Run)
- From the File New dialog box, you can access the:
  - New Run screen
  - New Sequence screen
- From the File Open dialog box, you can access the:
  - Run screen
  - Sequence screen
  - Chemistry screen
- From the Run screen, you can access the:
  - Associated Chemistry screen
  - Associated Sequence screen
  - Monitor screen
- From the Chemistry screen, you can access the:
  - Module screen
  - Cycle screen
  - Functions screen
  - Default Set screen
  - Chemistry Information screen

- From the Lab screen, you can access the:
  - Run screen (the Run sent most recently to the 433A)
  - Chemistry screen (the chemistry sent most recently to the 433A)
  - Text box to send messages to the Log
- Changes made in the Dictionary screen are automatically displayed in the:
  - Sequence screen
  - Compound palette
  - Chemistry screen
  - Run screen

Software and Hardware Limitations

C

This section describes the limitations of the module, cycle, run cycle changes, and sequence that are inherent in the SynthAssist® Software Version 3.1 and the ABI 433A Peptide Synthesizer.

Functions

A function can control a maximum of six valves.

Modules

A module can contain a maximum of 99 steps.

Cycles

A cycle can contain a maximum of 20 modules.

Cycles Changes in a Run

A Run can contain a maximum of 30 distinct cycles changes.

Bar Code Limit

A maximum of 255 bar codes can be sent to or stored in the 433A instrument.

- For Single Coupling (one cartridge per residue) the maximum sequence length in a Run file is 255 residues.
- For Double Coupling (two cartridges per residue) the maximum sequence length in a Run file is 127 residues.
- For 1.0 mMole runs (three cartridges per residue) the maximum sequence length in a Run file is 85 residues.

Note: The three length limits above arise from the bar-code memory capacity in the 433A instrument. The practical limits on the length of a peptide sequence in a synthesis are much lower and they arise from the volume capacity of the existing reaction vessels: 8 mL, 38 mL, or 55 mL. For example, a 1.0-mM synthesis in the large reaction vessel will accommodate a sequence length of approximately 20 residues, at which point the peptide-resin volume is approaching the capacity of the reaction vessel.

Software Warranty Information

D

This appendix covers:

Computer Configuration

Applied Biosystems supplies or recommends certain configurations of computer hardware, software, and peripherals for use with its instrumentation. Applied Biosystems reserves the right to decline support for or impose extra charges for supporting nonstandard computer configurations or components that have not been supplied or authorized by Applied Biosystems. Applied Biosystems also reserves the right to require that computer hardware and software be restored to the standard configuration prior to providing service or technical support.

Limited Product Warranty

Limited Warranty

Applied Biosystems warrants that for a period of ninety (90) days from the date the warranty period begins, its SynthAssist® 3.1 software will perform substantially in accordance with the functions and features described in its accompanying documentation when properly installed on the instrument system for which it is designated, and that for a period of ninety (90) days from the date the warranty period begins, the tapes, diskettes, or other media bearing the software product will be free of defects in materials and workmanship under normal use. If buyer believes that it has discovered a failure of the software to satisfy the foregoing warranty, and if buyer notifies Applied Biosystems of such failure in writing during the ninety (90) day warranty period, and if Applied Biosystems is able to reliably reproduce such failure, then Applied Biosystems, at its sole option, will either (i) provide any software corrections or “bug-fixes” of the identified failure, if and when they become commercially available, to buyer free of charge, or (ii) notify buyer that Applied Biosystems will accept a return of the software from the buyer and, upon such return and removal of the software from buyer's systems, terminate the license to use the software and refund the buyer's purchase price for the software. If there is a defect in the media covered by the above warranty and the media is returned to Applied Biosystems within the ninety (90) day warranty period, Applied Biosystems will replace the defective media. Applied Biosystems does not warrant that the software will meet buyer's requirements or conform exactly to its documentation, or that operation of the software will be uninterrupted or error free.

Warranty Period Effective Date

Any applicable warranty period under these sections begins on the earlier of the date of installation or ninety (90) days from the date of shipment for software installed by Applied Biosystems personnel. For all software installed by the buyer or anyone other than Applied Biosystems, the applicable warranty period begins the date the software is delivered to the buyer.

Warranty Claims

Warranty claims must be made within the applicable warranty period.

Warranty Exceptions

The above warranties do not apply to defects resulting from misuse, neglect, or accident, including without limitation: operation outside of the environmental or use specifications, or not in conformance with the instructions for the instrument system, software, or accessories; improper or inadequate maintenance by the user; installation of software or interfacing, or use in combination with software or products, not supplied or authorized by Applied Biosystems; and modification or repair of the product not authorized by Applied Biosystems.

The foregoing provisions set forth Applied Biosystems' sole and exclusive representations, warranties, and obligations with respect to its products, and Applied Biosystems makes no other warranty of any kind whatsoever, expressed or implied, including without limitation, warranties of merchantability and fitness for a particular purpose, whether arising from a statute or otherwise in law or from a course of dealing or usage of trade, all of which are expressly disclaimed.

Warranty Limitations

The remedies provided herein are the buyer's sole and exclusive remedies. Without limiting the generality of the foregoing, in no event shall Applied Biosystems be liable, whether in contract, tort, warranty, or under any statute (including without limitation any trade practice, unfair competition, or other statute of similar import) or on any other basis, for direct, indirect, punitive, incidental, multiple, consequential, or special damages sustained by the buyer or any other person or entity, whether or not foreseeable and whether or not Applied Biosystems is advised of the possibility of such damages, including without limitation, damages arising from or related to loss of use, loss of data, failure or interruption in the operation of any equipment or software, delay in repair or replacement, or for loss of revenue or profits, loss of good will, loss of business, or other financial loss or personal injury or property damage.

No agent, employee, or representative of Applied Biosystems has any authority to modify the terms of this Limited Warranty Statement or to bind Applied Biosystems to any affirmation, representation, or warranty concerning the product that is not contained in this Limited Warranty Statement, and any such modification, affirmation, representation, or warranty made by any agent, employee, or representative of Applied Biosystems will not be binding on Applied Biosystems unless in a writing signed by an executive officer of Applied Biosystems.

This warranty is limited to the buyer of the product from Applied Biosystems and is not transferable.

Some countries or jurisdictions limit the scope of or preclude limitations or exclusion of warranties, of liability, such as liability for gross negligence or willful misconduct, or of remedies or damages, as or to the extent set forth above. In such countries and jurisdictions, the limitation or exclusion of warranties, liability, remedies or damages set forth above shall apply to the fullest extent permitted by law, and shall not apply to the extent prohibited by law.

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