Implementing ACD/Automation Server Processing with Micro Flow-Injection and Open-Access NMR

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Amgen has been using ACD/Labs software as the default NMR processing tool for all chemists in discovery and development for over 12 years.

NMR data acquisition is handled with an in-house sample submittal tool, followed by manual processing with ACD/Labs - by the chemists.

ACD/Automation Server was installed in 2011 for processing all samples submitted to our High-Throughput Purification Platform - for structure verification preceding submission to sample repository.

Automation Server has been integrated into all Open-Access NMR for processing and distributing data files for all walk-up NMR users.

Automation Server has created a pathway for building a searchable database for all NMR sample submissions.
Automating NMR Systems at Amgen

High-Throughput Purification Platform (HTPP)

- Develop an automated **software platform** for acquiring, processing and distributing NMR data for structure verification in support of the High-Throughput Purification / sample registration process.

- Develop a robust **hardware platform** that can utilize existing HTPPP protocol while improving our operational efficiency:
  - reduce sample size and consumable costs.
  - reduce setup time for data collection and processing.
  - reduce time and effort for data review by the chemist.
NMR Support for the HTPP Platform

1. **Hardware Solution** – micro-flow NMR readily supports small sample sizes in 96-well plate format (25 µL/well).

2. **Software Solution** – One-Minute NMR from Protasis provides automated sample submissions using SD files generated by Pipeline Pilot following purification.

3. **Data Processing Solution** – ACD/Labs Automation Server software is used for processing and distributing NMR data - minimizes processing and analysis time for the chemist.

4. **Data Archiving Solution** – individual plate and single sample database files are being created for each chemist in *nd9* format - full Oracle DB is work in progress.
Hardware Solution - Flow Injection NMR

- Running High-Throughput Flow-NMR Samples
  
  Flow-NMR systems can be tailored to accommodate different sample streams as a means of improving the sensitivity for a given analysis.

- Cell volumes available for Bruker “Pass-Through” Cryoprobe
  
  Flow Cell Volumes: 10 30 60 120 µL

  High concentration Low Concentration

- RT Flow-Systems – fully integrated systems are available from Protasis, Bruker, and Agilent using probes with cell volumes ranging from 5-120 µL.

- Cryo-Flow Probes - provide better sensitivity using 10-60 µL flow cells, convenient sample handling - user customized systems through Protasis.
Flow Injection NMR at Amgen

**NMR Instrumentation** – two flow systems using 96-well plate format or vials.

1. **Bruker 500** - RT Protasis ICG probe (10 µL flow cell), LEAP liquid handler, dual pump Protasis Discovery Tower, solvent conditioner - uses One-Minute NMR software interfaced with ACD Automation Server processing.

2. **Bruker 600** with a 5 mm TCI cryoprobe using a variable flow cell (VFC) insert with a LEAP liquid handler, using a one pump Protasis flow system and solvent conditioner, running One-Minute NMR software and ACD automation server.

**Intended Use**

**HTPP- NMR:** samples from high-throughput purification - as a structural-confirmation step for the compound registration process prior to submission to chemical repository.

**Quantitative Analysis:** all HT-NMR samples are run using Q-NMR (with Digital-Eretic referencing) to give estimated concentrations at time of submission.

**Screening:** flow-NMR methods are also being developed as a tool for Fragment-Based screening applications in aqueous media.
**HTPP Process** - we developed an **automated Mass-Directed purification** process to aid chemists in their efforts to design and synthesize new therapeutic agents.

Chemists employ parallel synthesis to make focused libraries which are purified and characterized using LC-MS and $^1$H NMR using Flow-NMR techniques.

Purified materials are provided in Sample Bank vials and processed NMR data is provided to chemists in a convenient electronic format, sent directly to their e-LN.
Sample collection is triggered by Mass Spec and the selected product is dried, weighed and formatted into Sample-Bank and solubility vials and two 96-well plates are prepared in automation using a Tecan Evo robot.
Flow-NMR technology is reliable and reproducible with minimal downtime in practice: more than 6000 samples were run on this system in 2013.

Samples are dissolved in 25 µL of dmsot-d<sub>6</sub> (~20 mM), covered and placed in LEAP.

Samples and solvent are kept dry with closed system, using dry gas blanket.

Greatly reduced sample requirements - 15 µL injections used to acquire NMR.

Protasis NMR Probe with 10 µL flow-cell
Pipeline Pilot is a query tool that allows us to download sample submittal metadata for HTPP-NMR plates submitted for NMR analysis. The resulting SD file is used to collect and process the NMR data.
Running HTPP-NMR in Automation

NMR is Setup by Shooting a Barcode

- Reads SD File from Pipeline Pilot
- SD File

One-Minute NMR

- e-Mail is sent to NMR group when new plate is ready
- SD file is imported directly into One-Minute NMR
- Generates a sample queue
- Click “Queue Run” to start NMR for the entire plate

Click Run
SD files are used to run NMR data collection - this process also available for Single sample submissions

- The chemist creates a SD file for sample from their e-LN
- Pipeline-Pilot creates final format
- One plate can be composed of samples from multiple users
- The SD file sets up queue for NMR acquisition and automated processing
- Process accepts single samples as well as libraries.

Single samples can be dropped off for purification and NMR
ACD/Labs software pulls out individual SD files for each sample and it this is sent to the NMR where it is embedded in the NMR data tree.

Example: 12345-01-01.sdf → copied to Bruker data folder

When NMR is finished, data file is sent to sweep directory (or Data Drop-Box) and this triggers auto-processing with ACD/Automation Server software.

Automation Server processes, archives and distributes the data to the chemist.

Output → Raw data, processed data, database file, and pdf report.
ACD/Labs Output: the Processed-Data Tree

Processed Data stored on central file server

3 Folders are Created

1. **esp** files - processed spectra with integrals, peak picking and embedded structures. Linked to ACD/Spectrus Processor.

2. **plate-database** - contains a NMR database for each plate, also linked to ACD/Spectrus Database Manager.

3. **reports** - 1 or 4 page **pdf** plots for each sample and combined report for each plate (for rapid review).
esp file - Processed spectrum viewed with ACD/Spec Manager
Example Output: NMR Database Files (nd9)

- In Table View

Click on spectrum to start processor

Browse with scroll tab
NMR Database File - in Plate View

Click on this spectrum -
starts ACD/Spectrus Processor

Click on a well - shows the processed spectrum
Example Four Page Report – sent to e-LN Inbox

Data summary for notebook in journal format
Four Page Report – Expansions on pages 2/3
Reports are deposited in the chemists reports folder and a copy is mailed to the inbox of their Electronic Notebook.

Review of the pdf report often can be used for final review without the need for reprocessing the data.

A combined pdf report is created per plate - can review all samples in one file.

This process has been expanded to accommodate single sample submissions Single sample submissions are supported – libraries are no longer required.
Improvements in Flow-Injection NMR

The flow system runs 1D proton spectra from 96-well plates with minimal human intervention at a rate of 5 samples/hour.

Data acquisition and processing steps have been automated using a barcode scanner and ACD/Automation Server software.

Areas of Improvement

- The cycle time for a 20 mM sample is long (~12 min/sample).
- S/N for 2D spectra is low - g-HSQCdept spectra take over 1 hour.
- S/N improvements can be made by using cryoprobe technology.
- Variable volume flow cells can be used to suit the sample stream.
600 MHz Cryo-Flow NMR System with Protasis Hardware

- Commercial flow cryoprobe system using a 600 MHz Bruker with a 30 μL flow-cell insert on Cryo-Fit pole

- Utilizes a Protasis solvent pump, LEAP liquid handler and OM-NMR software

- Optimized injection volume is 25 μL

- S/N testing shows that this system has ~20X better sensitivity than current 500 MHz systems

- 25 μL of 20 mM Quinidine gives proton and g-HSQCdept spectra in 10 minutes

- Good data has been obtained on 0.5 μmol samples of complex mixtures

Flow Cell

- 30 μL (active volume)
- 60 μL (total volume)

Improved Sample Throughput
Bruker Cryo-Fit flow-cell attachment for a standard 5-mm CryoProbes with a pass-through design

- Flow cell is on a pole that is inserted down the magnet bore
- Samples pushed in from the bottom and exit to waste out top of pole
- PEEK tubing is force fit into the ends of the flow cell – uses 1/32” OD
- Tubing is easily replaced
- Various flow cells are available
Variable Flow Cell Inserts – Replace Cryo-Fit

- Commercial flow cryoprobe system uses a Cryo-Fit pole with a fixed flow cell.

- Simple flow-cell inserts can be made with tube adapters that attach to the flow cell.

- Flow insert fits into spinner which easily drops into the pass-through cryoprobe.

- Different cell volumes can be used to provide interchangeable low cost inserts.

- Available sizes are 10, 30, 60 and 120 µL.

- Can be switched easily to best match the volume of the analytical sample stream.
Example Proton Spectrum of Quinidine
15 µL Injection on the 500 RT Flow-Probe

20 mM Quinidine in dmso-d$_6$

8 Scans  1 min
S/N = 95:1
Proton Spectrum of Quinidine 25 µL injection using a 600 Cryo-Flow Probe

20 mM Quinidine in dmso-d$_6$

8 Scans 1 min
S/N = 3292:1

dmso-d$_6$
1D Proton + HSQCdept of 20 mM Quinidine
25 µL injection in a 600 Cryo-Flow Probe

20 mM Quinidine in dms-o-d₆

1 Scan HSQC = 5 min
600 Cryo-Flow Probe with a Protasis Delivery System

- Cryo-flow probe has about 20X the S/N of the RT Protasis probe*

<table>
<thead>
<tr>
<th>Flow System</th>
<th>Sample Volume</th>
<th>NS</th>
<th>S/N Ratio</th>
<th>Scale Factor</th>
<th>Time per Sample</th>
<th>Samples per Hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 RT-Probe (10 µL)</td>
<td>15 µL</td>
<td>256</td>
<td>528:1</td>
<td>1</td>
<td>23 min</td>
<td>~3</td>
</tr>
<tr>
<td>600 Cryoprobe</td>
<td>25 µL</td>
<td>256</td>
<td>17,436:1</td>
<td>20X</td>
<td>25 min</td>
<td>~2</td>
</tr>
<tr>
<td>600 Cryoprobe</td>
<td>25 µL</td>
<td>8</td>
<td>3,292:1</td>
<td>3.7X</td>
<td>3 min</td>
<td>20</td>
</tr>
</tbody>
</table>

*The S/N for 20 mM Quinidine in DMSO-d₆ values are scaled for the quantity of material injected (raw S/N is multiplied by 15/25). This is a rough estimate.
Improving NMR Operations

Updating Open-Access NMR

- **Update Open-Access NMR** - to interface with ACD/Automation Server processing tools that were developed for HTPP-NMR.

- **Update Submittal Tools** - custom web application is used to submit samples to the Open-Access NMR systems.
  
  - *Chemdaw* structures need to be added to the sample submission step in order to take full advantage of the ACD automation tools.
  
  - Peak-picking, auto-scaling, integration and plotting format are improved when chemical structures are included with submission.

  - **ACD/Labs software** creates a relational database - provides tools for searching spectra based on full or partial structural information.
ACD/Automation Server added to Open-Access NMR

- Fully processes data
- Outputs pdf Reports
- Relational Database

Processing Done by ACD/Labs
- Integrated NMR output
- 1 or 4 page pdf reports
- Structures included
- NMR database created
- Can search by structure
Modified Sample Submission Web Tool

Adding Structures to Sample Submissions

- Add Chemdraw Plug-in to Capture Structure.
- Structure saved as mol file and sent to NMR along with existing metadata to run instrument.
- Completed spectra sent to ACD/Labs sweep directory.
- Auto-processing and data distribution is the same as performed with the current HTPP-NMR process.
- All reports, processed data and database files are generated automatically.
ACD/Labs Automated Processing – offers a number of improvements.

- Formatted *pdf* reports with structures are created for fast data review.
- Data reprocessing is often not required – ID made from *pdf* output.
- Processed data is provided directly in 2 ACD formats – *esp* and *jdx*
  
  - Peak Picking to list position of NMR signals – *included*
  - Signal Integration to check proton count – *included*
  - Formatted output for records sent to e-LN – *included*
  - Searchable database provided for each user – *included*

- Automated processing and plotting – can save the chemists time.
- NMR data for related compounds can be quickly compared for ID work.
- Recently implemented at all sites using a *Structures* folders.
Example of a Correct vs. Incorrect Structure

Having the structure displayed on the spectrum makes it easy to identify the correct structure.

Proton NMR - aromatic region
New Files in Open-Access ACD/Labs Processing

processed-data on file server

2 New Folders Created

1. **open-access-database** - contains NMR database of each sample submitted, all spectra can be viewed with ACD/Spectrus DB Manager.

2. **structures** - folder where Chemdraw structures are stored before submitting a sample. Structures are attached during auto-processing.
ACD/Labs Processing in Open-Access

Structure captured from file

\[ ^1H \text{NMR (400 MHz, DMSO-d}_6\] \delta ppm 1.32 - 1.53 (m, 3 H) 1.69 (br. s., 1 H) 1.83 - 1.95 (m, 1 H) 2.14 - 2.23 (m, 1 H) 2.52 - 2.73 (m, 3 H) 2.92 - 3.15 (m, 2 H) 3.88 - 3.91 (m, 3 H) 5.03 - 5.11 (m, 2 H) 5.25 - 5.32 (m, 1 H) 5.65 (d, J=4.46 Hz, 1 H) 6.04 - 6.14 (m, 1 H) 7.38 (dd, J=9.17, 2.70 Hz, 1 H) 7.45 (s, 1 H) 7.50 (d, J=4.59 Hz, 1 H) 7.92 (d, J=9.12 Hz, 1 H) 8.68 (d, J=4.46 Hz, 1 H)
Open-Access NMR - adding an Integrated Data Platform

- **Open-Access submissions** – adding structures provides a common output format that can be used to build a structure searchable database for all NMR data.

- **NMR Database** - can be linked to e-Notebook and In-house sample management tools.

- **Integrated Search Tools** - can be deployed via web based search tools.

- **NMR Database** - can be viewed with Spectrus which provides searching and viewing capabilities.

- **Adding Assignments to the Database** - improve the accuracy of ACD/Labs Chemical Shift prediction tools.
NMR Database Consolidation

- **ACD/Labs Enterprise Database** software can be utilized to manage all NMR data – from fully assigned spectra to Open-Access data.

- **Database Integration** is supported by the deployment of Spectrus DB – updated version available to all chemists at Amgen.

- **Expert NMR Database** is being built for the NMR spectroscopy groups with access across all discovery and development groups.

- **HTPP-NMR Database** is being built for all samples being registered in or sample repository – Sample Bank.

- **Open-Access Database** is being built for storing all self service NMR data, with and without attached structures.

- ACD/Labs software will provide pathway for **future integration** with other analytical data across Amgen (LC/MS etc.).
Example of ACD/Spectrus DB Search Tools
Comparing Spectra from Database Files
- most common use of database for general users

Comparing two spectra is easy with ACD/Labs database tools
Conclusions

Flow NMR offers a number of advantages over tube-based methods, particularly with ease of use and reproducibility.

Cryoprobe technology offers a great advantage in terms of S/N and sample throughput in flow NMR - 2D analysis can be done much faster.

Variable flow cell inserts can be used to optimize the S/N and minimize acquisition time when samples with different composition and concentration are provided for NMR analysis.

ACD/Labs software has proven to be a versatile and reliable tool for processing NMR spectra in automation – using both tube and flow based NMR techniques.

NMR database will provide a fully integrated into the sample management system to improve efficiency of NMR analysis.
Thanks go to …

- Ashley Nichols \(\text{ACD/Labs}\)
- Paul Krolakowski
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- Ken Charest
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- Justin Provchyy
- Peter Grandsard
- Dave Strand, Protasis
- Rob Krull, Bruker BioSpin
- Dave Snyderman
- Dave Adams
- Igor Teslya
- Patrick Wheeler
ACD/Labs Software tools are used throughout R&D at Amgen - both in Chemical Discovery and Development

Partial listing of ACD/Labs software available to the chemistry community at Amgen.

ACD/Spectrus Processor is the default NMR data processing and viewing tool for Open-Access NMR at Amgen.

- Newest version released in Q4/2013.
- Large training footprint is in place.
Q-NMR Reproducibility with the Flow-CP

Repeat injections from different wells

Calculated Concentrations - 25 µL injections of 20 mM Quinidin with DE referencing

Ave Conc = 19.13 mM
Std Dev = 0.10 mM (0.5%)

Recovery = 95.7%
Stated Purity = 98.0%

Q-NMR using Protasis System / LEAP Autosampler is very Reproducible