



ACD/Labs

Visionary Software



Advancing Research

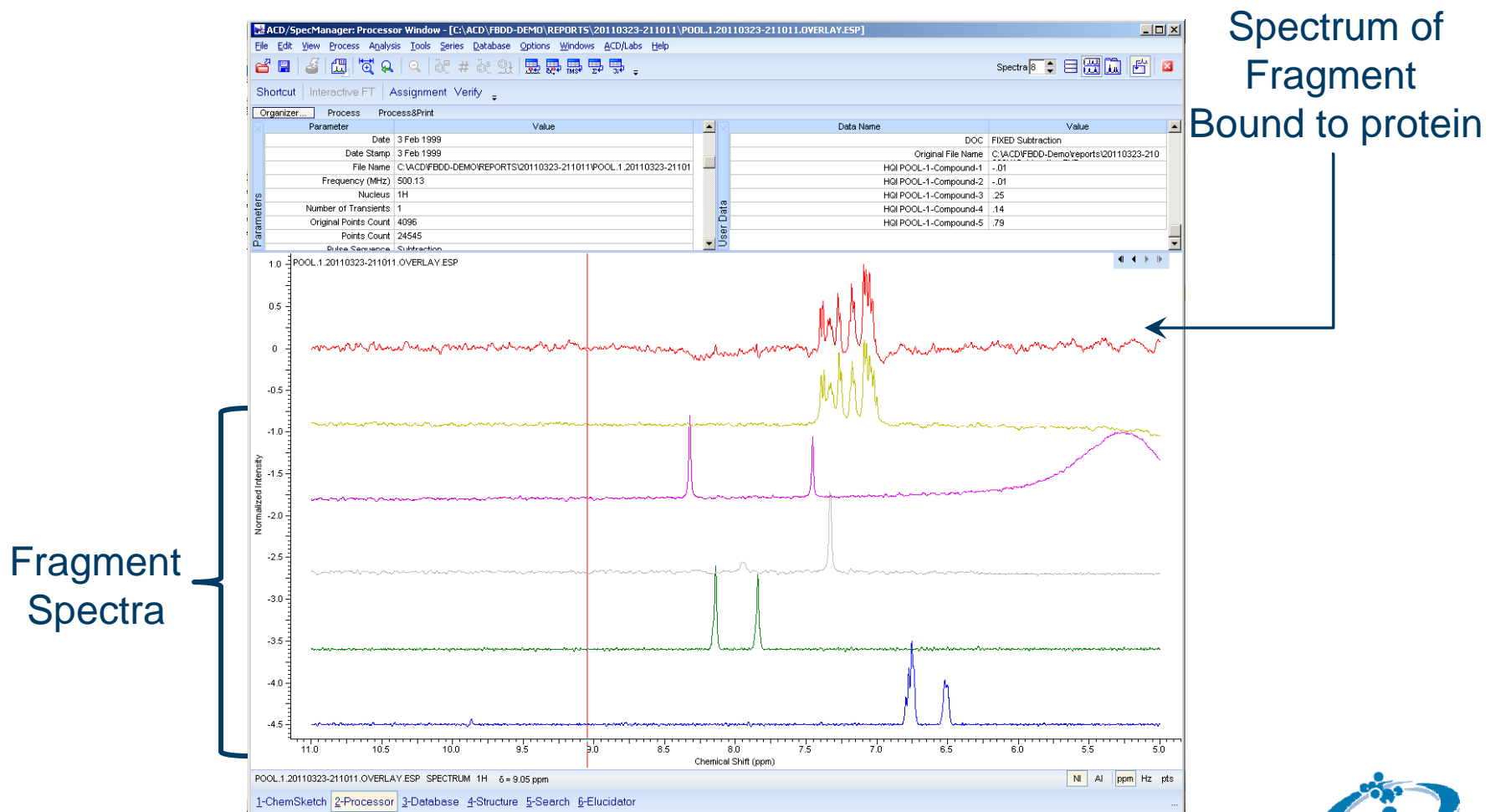
Fragment Based Drug Screening: A study in visualization and processing

Agenda

- Look and feel
- Fragment based screening: a short primer
- Bits and pieces
- Outputs, options, and usage
- Conclusions

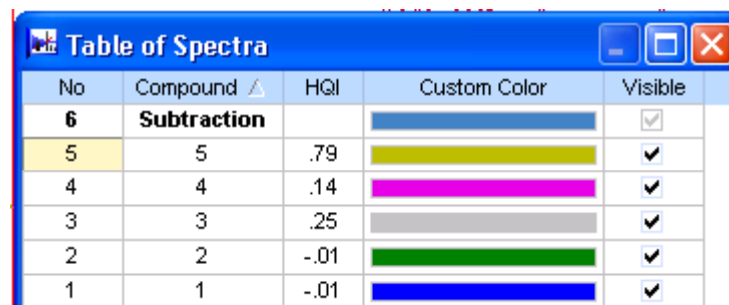


Visual Review of Data



Quantitative Review

- Calculate similarity between “bound” spectrum and each ligand
- Use “Hit Quality Index” to quantify the similarity



No	Compound Δ	HQI	Custom Color	Visible
6	Subtraction			<input checked="" type="checkbox"/>
5	5	.79		<input checked="" type="checkbox"/>
4	4	.14		<input checked="" type="checkbox"/>
3	3	.25		<input checked="" type="checkbox"/>
2	2	-.01		<input checked="" type="checkbox"/>
1	1	-.01		<input checked="" type="checkbox"/>



What is FBDD?

- Fragments: smaller space to span
- Proteins have multiple active sites
- Searching for fragments that can be combined to interact with proteins
- Five or more fragments per well
- Five, ten, or more plates in fragment library
- Each protein screened against the library



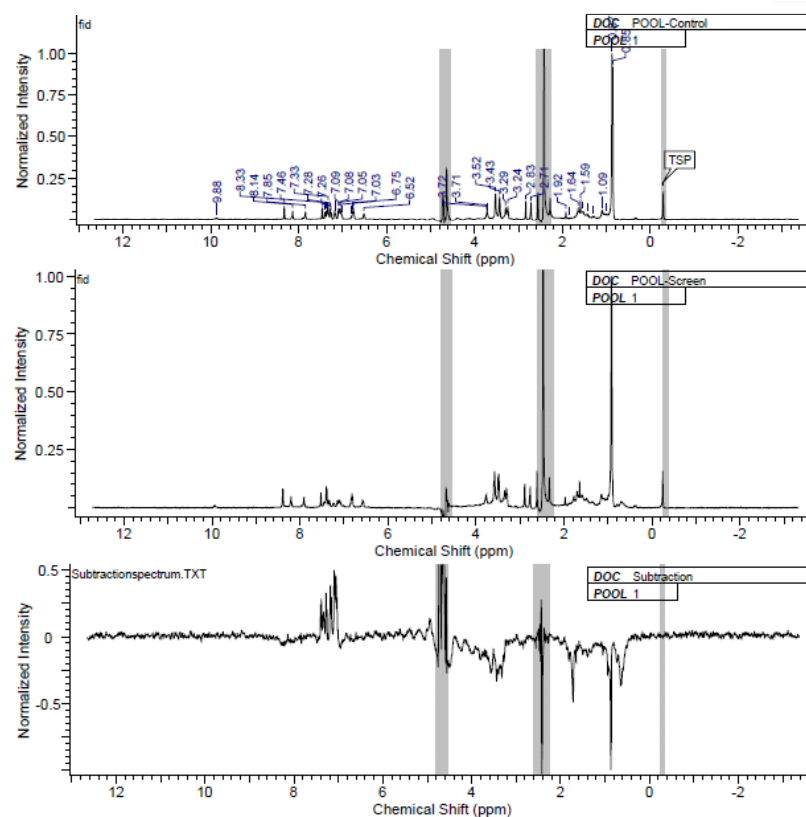
NMR Screening Options

- Saturation Transfer Difference (STD): saturate protein with rf – signals are transferred via spin diffusion to bound ligands, which appear in spectra.
- WaterLOGSY: magnetization transfer from water.
- TINS: ligand signal suppressed by shortened T_2 – protein target coupled to solid phase.
- T2-Based Screening – Ligand relaxation is affected by binding to protein and thus signals are attenuated during a spin-lock period.



NMR Screening Mechanics

- Take spectrum without interaction “turned on”
- Take spectrum with interaction in place
- Subtract to find NMR signal of “bound” ligands



ACD Scripting

```
fbddUtils.cpp (C:\ACD\FBDD-Demo\bin) ((1) of 6) - GVIM
File Edit Tools Syntax Buffers Window Help
|A\F\b\specDbUtils.cpp |A\F\b\acdConfig.cpp |A\F\b\commonUtils.cpp |A\F\b\fbdd.cpp |A\F\b\fbddUtils.cpp |A\F\b\showOverlay.cpp
45
46 /*
47 * Function: subtractSpecDocs
48 * Parameters:
49 *   pool: the spectrum containing the pool
50 *   hit: spectrum of ligands bound to protein
51 * Method:
52 *   Grab acquisition parameters to use in calculating spectral offsets and
53 *   such. Pull the data vectors from the two spectra. Calculate a common
54 *   scale. Subtract the two vectors. Create a new document from the
55 *   vectors.
56 * Returns:
57 *   Spectral document with subtracted spectrum.
58 */
59 TSpecDocument subtractSpecDocs( TSpecDocument pool, TSpecDocument hit ) {
60   string poolNuc = pool.GetParameter( "Nucleus" );
61   int poolOp = getParamInt( pool, "Original Points Count" );
62   float poolSw = getParamFloat( pool, "Sweep Width (Hz)" );
63   float poolOs = getParamFloat( pool, "Spectrum Offset (Hz)" );
64   float poolTemp = getParamFloat( pool, "Temperature (degree C)" );
65   float poolSF = getParamFloat( pool, "Frequency (MHz)" );
66   string poolName = pool.GetUserData( poolField );
67   logText( "pool sf: " + fts(poolSF) + " nuc: " + poolNuc + " op: " + its(poolOp) + " s
w: " + fts(poolSw) + " os: " + fts(poolOs) + " temp: " + fts(poolTemp) );
68
69   string hitNuc = hit.GetParameter( "Nucleus" );
70   int hitOp = getParamInt( hit, "Original Points Count" );
71   float hitSw = getParamFloat( hit, "Sweep Width (Hz)" );
72   float hitOs = getParamFloat( hit, "Spectrum Offset (Hz)" );
73   float hitTemp = getParamFloat( hit, "Temperature (degree C)" );
74   float hitSF = getParamFloat( hit, "Frequency (MHz)" );
75   logText( "hit sf: " + fts(hitSF) + " nuc: " + hitNuc + " op: " + its(hitOp) + " sw: "
+ fts(hitSw) + " os: " + fts(hitOs) + " temp: " + fts(hitTemp) );
76
77   TVector newX = TVector.Create();
78   TVector newY1 = TVector.Create();
79   TVector newY2 = TVector.Create();
80   TVector hitX = TVector.Create();
81   TVector hitY = TVector.Create();
82   TVector poolX = TVector.Create();
83   TVector poolY = TVector.Create();
84
85   logText( "Retrieve Vectors" );
86   pool.GetDataPoints( poolX, poolY, true );
87   hit.GetDataPoints( hitX, hitY, true );
88
89   int poolPeakPt = findPeakPoint( poolX, poolY, poolSF, 0.0, -0.3 );
90   logText( "poolPeakPt: " + its(poolPeakPt) + " x: " + fts(poolX[poolPeakPt]) + " y: " +
fts(freqToPpm(poolX[poolPeakPt], poolSF, 0.0)) + " y: " + fts(poolY[poolPeakPt]) );
91
92   int hitPeakPt = findPeakPoint( hitX, hitY, hitSF, 0.0, -0.3 );
93   logText( "hitPeakPt: " + its(hitPeakPt) + " x: " + fts(hitX[hitPeakPt]) + " y: " + fts(
freqToPpm(hitX[hitPeakPt], hitSF, 0.0)) + " y: " + fts(hitY[hitPeakPt]) );
94
95   float os = poolX[poolPeakPt] - hitX[hitPeakPt];
96   logText( "adjust offset: " + fts(os) + " x: " + fts(freqToPpm(os, hitSF, 0.0)) );
97   hitX.AddValue( poolX[poolPeakPt] - hitX[hitPeakPt] );
```

- Versatile environment to allow for custom data processing
- Hosted within ACD Automation products and now in ACD Desktop products, too



Subtraction in Scripting

- Pull raw data from spectra
- Reference and normalize each based on TSP peak
- Subtract point to point
- Store in ASCII format and load as subtracted spectrum



HQI in Scripting

- Truncate to window for calculation
- Pull raw data from spectra as vectors
- Harmonize both vectors
- Normalize each vector
- HQI is dot product



Alternatives

- Do subtraction by phase cycling
- Spectrometer can perform subtraction
- Use peak-by-peak accounting instead of HQI
- Renormalize HQI by ligand mass



Script Flow for FBDD

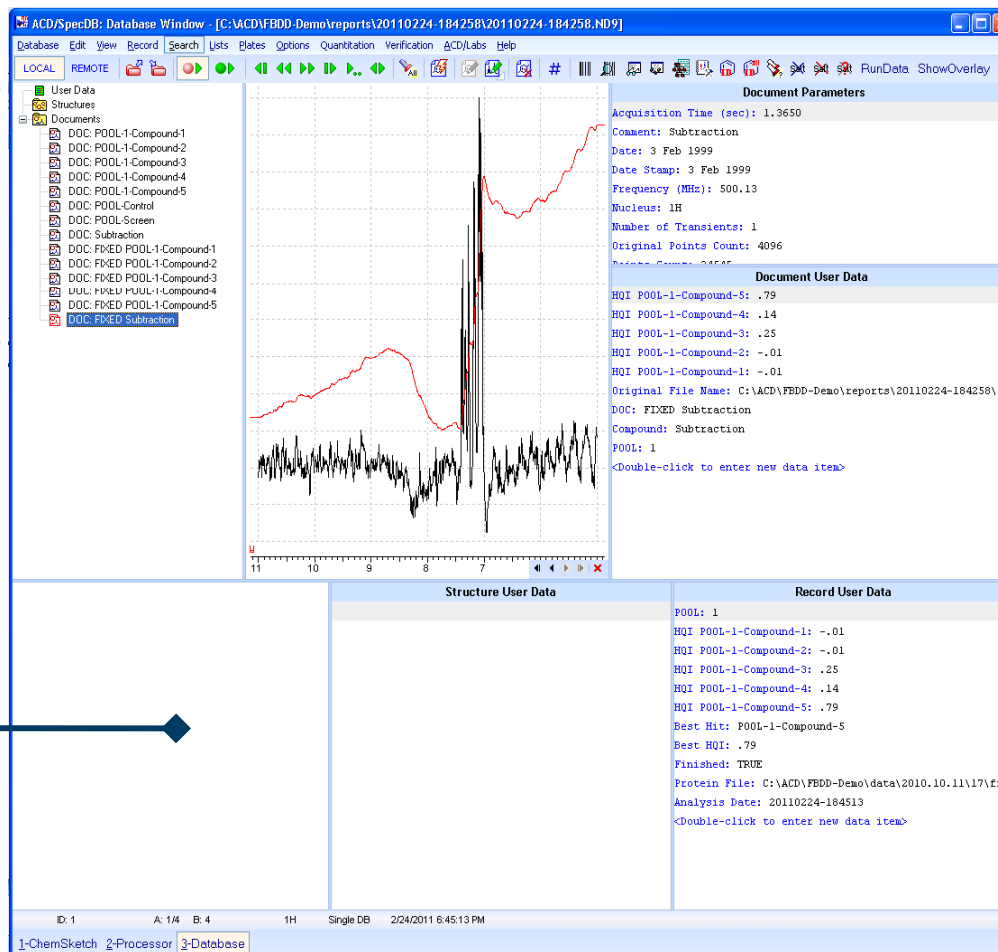
- Read in bound and unbound, calculate subtracted, and truncate to clear window
- Read in ligand spectra (or pull from library) and truncate to clear window
- Measure HQI for each truncated ligand spectrum against truncated subtracted spectrum



Analysis Output: ND9

Use document
Tree for quick
selection

Can
populate
Structures
from
Mol, SD,
database

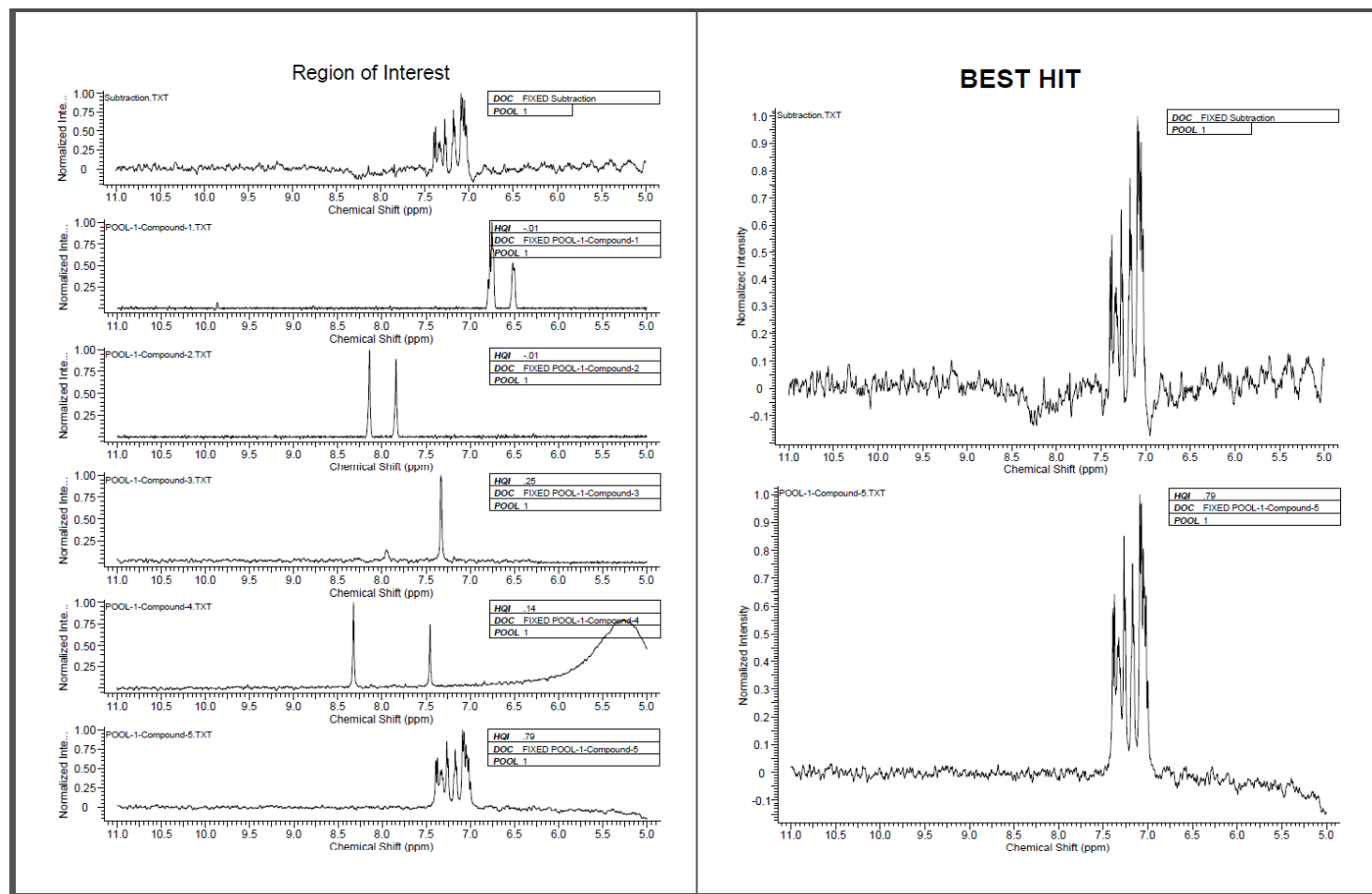


Summary
Of HQI



ACD/Labs

Analysis Output: PDF Report



Analysis Output: CSV Report

The screenshot shows an Excel spreadsheet with the following data:

	A	B	C	D	E	F	G	H	I	J	K	L
1	Pool	Cmpnd1	Cmpnd2	Cmpnd3	Cmpnd4	Cmpnd5	Best Compounds	Cmpnd Hits				
2	1	-0.01	-0.01	0.25	0.14	0.79	POOL-1-Compound-5	5				
3	2	0.11	0.56	0.3	0.17	0.15	POOL-2-Compound-2					
4	3	0.04	0.12	0.1	0.58	0.05	POOL-3-Compound-4					
5	4	0.02	-0.02	0.27	0.13	-0.01	POOL-4-Compound-3					

Well of Five compounds

HQI of each compound

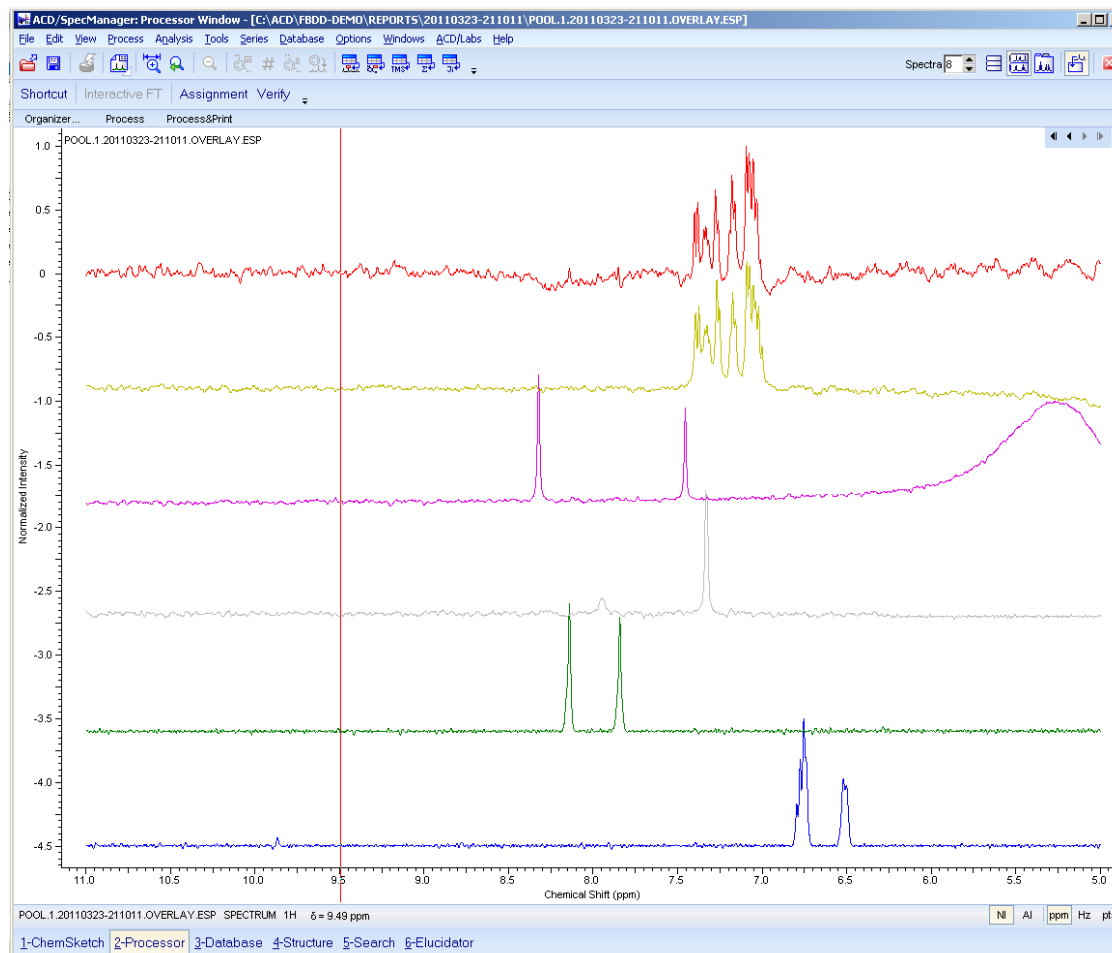
Compound in Well with Highest HQI

List of compounds with HQI above threshold

So... putting it all together

Subtracted
Well-bound ligand

Not bound



ACD/Labs

Future Work

- Improve subtraction algorithms: especially alignments and normalizations
- Evaluate alternative hit recognitions and quantifications
- Work with other screening methods

