Computational Spectral Analysis

PERCH NMR Software Course

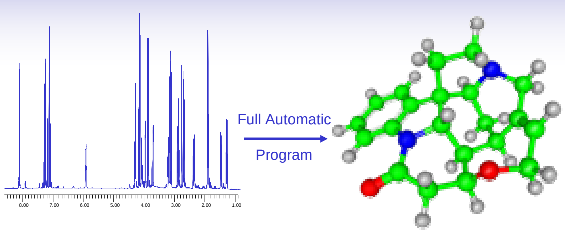
Turku - 16.- 17.5.2006

Why Computational Spectral Analysis?

- To obtain more accurate spectral parameters
- To better understand second-order spectra
- To analyze large spin-systems containing lots of coupling information
- **Can be automated!**

© 2001-2006 PERCH Solutions Ltd. – CSC/PERCH NMR Software Course, Turku 16.-17.5.2006

Every Chemist's Dream



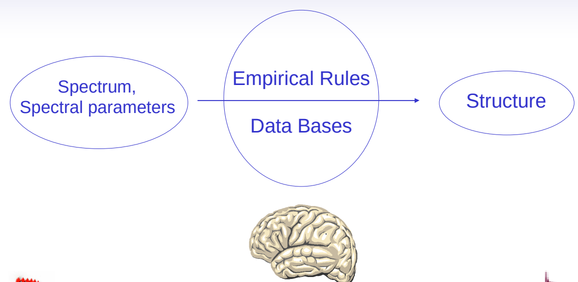
Spectrum

Structure

© 2001-2006 PERCH Solutions Ltd. – CSC/PERCH NMR Software Course, Turku 16.-17.5.2006

The Usual Way

Works reasonably for ^{13}C-spectra



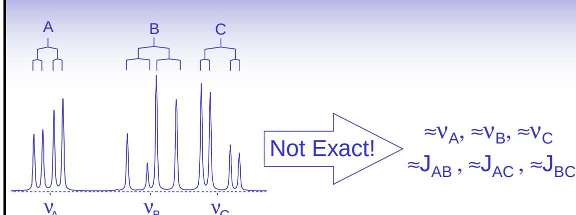
© 2001-2006 PERCH Solutions Ltd. – CSC/PERCH NMR Software Course, Turku 16.-17.5.2006

NMR Observables for structure and dynamics

Observable	Information
• Chemical shifts 1H,13C,15N,31P	Assignments, Secondary Structure
• J-couplings (through bond) 3J(HN,Hα), 3J(Hα, Hβ), …	Dihedral angles: Φ, χ, Karplus curves
• NOE (through space)	Interatomic distances (<5Å)
• Solvent exchange (HN)	Hydrogen bonds
• Relaxation / linewidths 1H,13C,15N	Mobility, dynamics conform./chem.exchange
• Residual dipolar couplings	Projection angles

© 2001-2006 PERCH Solutions Ltd. – CSC/PERCH NMR Software Course, Turku 16.-17.5.2006

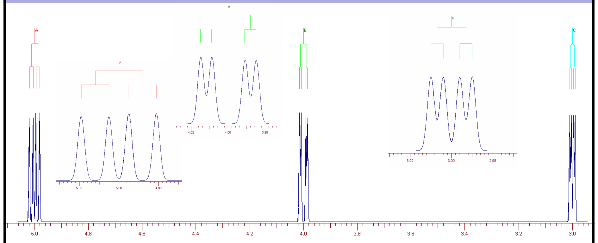
Problem



- Retrieved spectral parameter not exact!

© 2001-2006 PERCH Solutions Ltd. – CSC/PERCH NMR Software Course, Turku 16.-17.5.2006

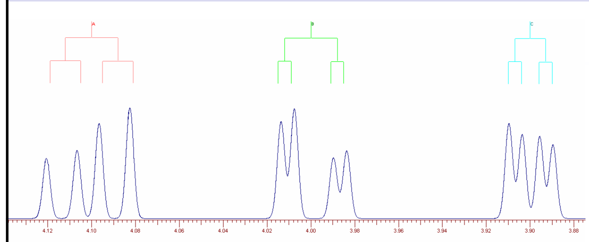
First Order



$\nu_A - \nu_B >> J_{AB}$

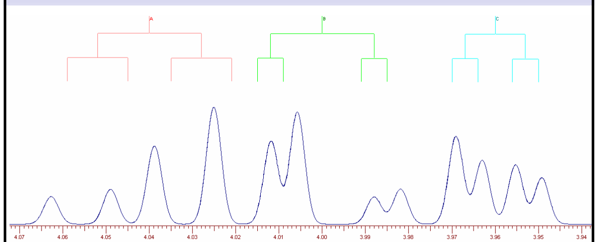
- ”First order” spectral parameters ”almost” exact!

”Slight” Second Order



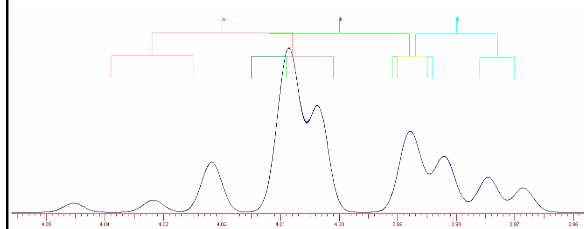
- ”First order” spectral parameters still good!

”Clearly” Second Order



- ”First order” spectral parameters pretty much wrong!

”Strong” Second Order

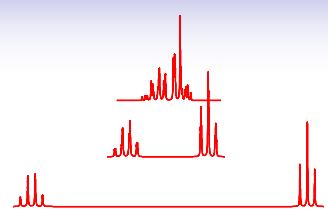


- ”First order” spectral parameters completely wrong!

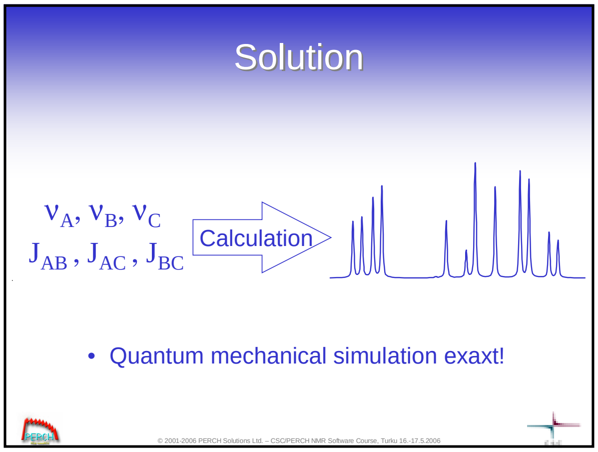
1.Order → 2.Order

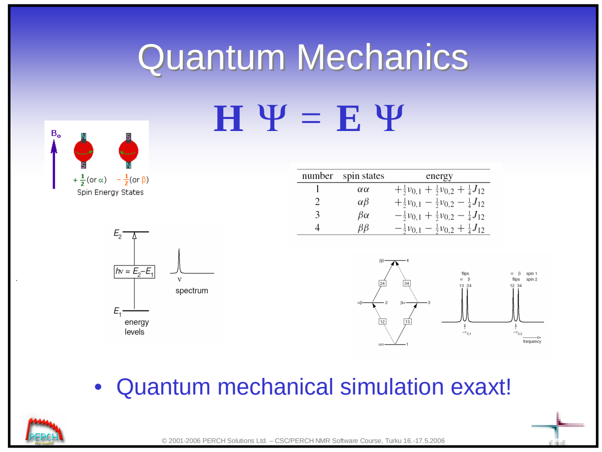
- First signal intensities change, then splittings and at last multiplet widths.
- Chemical shifts are at multiplet center.
- Splittings corresponding to coupling constants of coupled nuclei are slanted towards each other.
- Splittings correlate with the corresponding couplings. 2. Order equalizes splittings from different couplings.

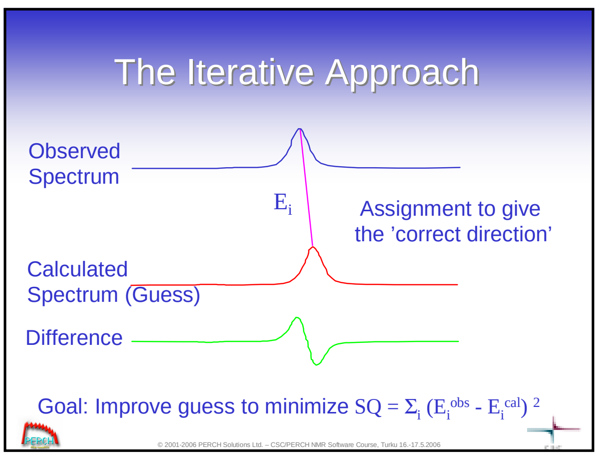
Pattern Recognition

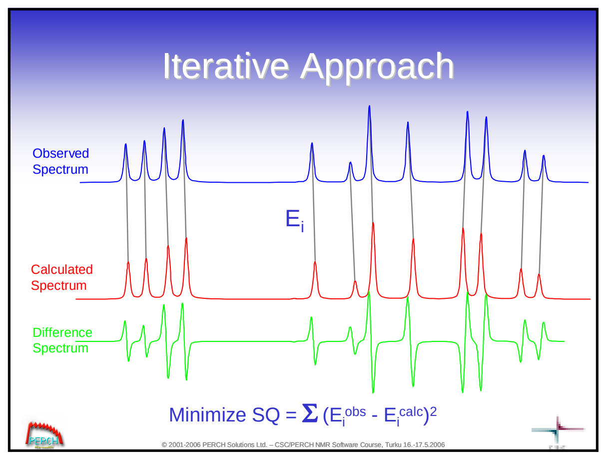


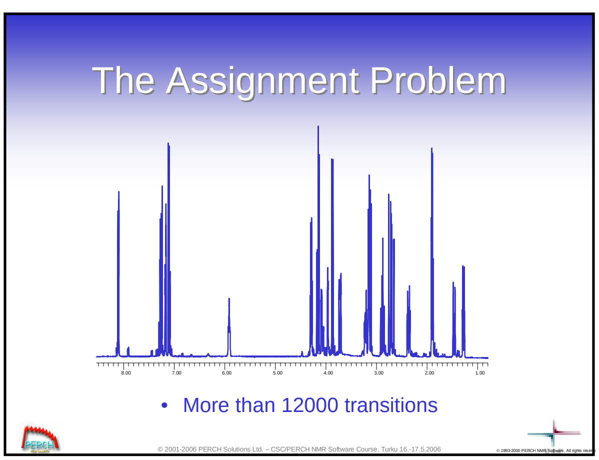
- Problematic with second order spectra
- A simple data base approach cannot work

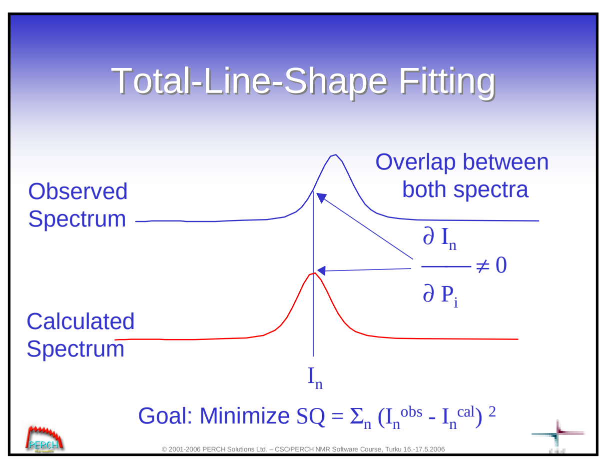

Solution
ν_A, ν_B, ν_C
J_{AB}, J_{AC}, J_{BC}
Calculation
• Quantum mechanical simulation exaxt!
© 2001-2006 PERCH Solutions Ltd. – CSC/PERCH NMR Software Course, Turku 16.-17.5.2006

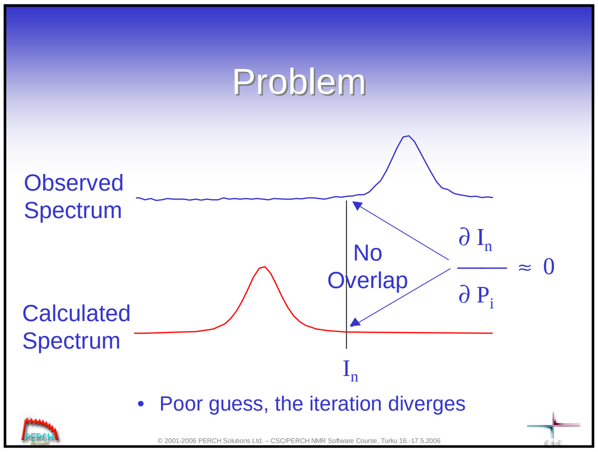

Quantum Mechanics
H Ψ = E Ψ
Spin Energy States
spectrum
energy levels
number spin states energy
• Quantum mechanical simulation exaxt!
© 2001-2006 PERCH Solutions Ltd. – CSC/PERCH NMR Software Course, Turku 16.-17.5.2006

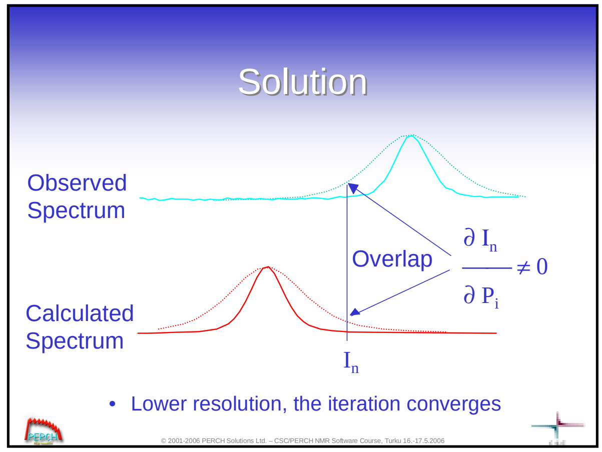

The Iterative Approach
Observed Spectrum
E_i
Assignment to give the 'correct direction'
Calculated Spectrum (Guess)
Difference
Goal: Improve guess to minimize $SQ = \Sigma_i (E_i^{obs} - E_i^{cal})^2$
© 2001-2006 PERCH Solutions Ltd. – CSC/PERCH NMR Software Course, Turku 16.-17.5.2006

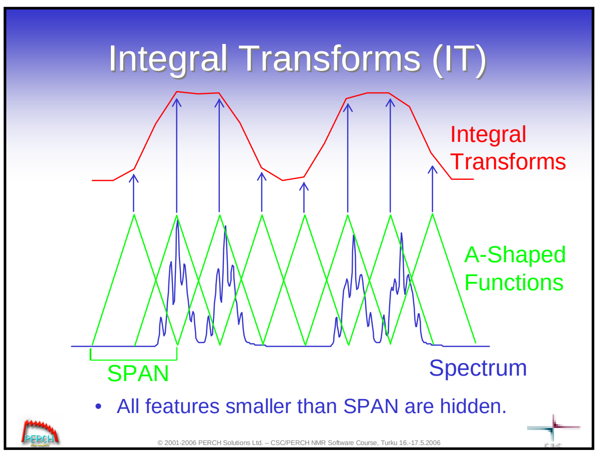

Iterative Approach
Observed Spectrum
E_i
Calculated Spectrum
Difference Spectrum
Minimize $SQ = \Sigma (E_i^{obs} - E_i^{calc})^2$
© 2001-2006 PERCH Solutions Ltd. – CSC/PERCH NMR Software Course, Turku 16.-17.5.2006

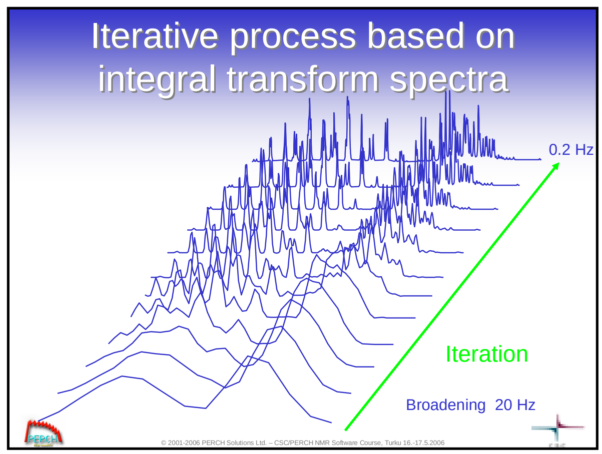

The Assignment Problem
• More than 12000 transitions
© 2001-2006 PERCH Solutions Ltd. – CSC/PERCH NMR Software Course, Turku 16.-17.5.2006

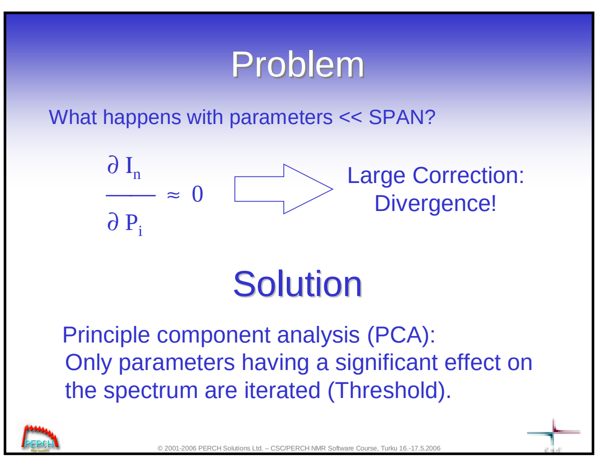

Total-Line-Shape Fitting
Observed Spectrum
Overlap between both spectra
$\frac{\partial I_n}{\partial P_i} \neq 0$
Calculated Spectrum
I_n
Goal: Minimize $SQ = \Sigma_n (I_n^{obs} - I_n^{cal})^2$
© 2001-2006 PERCH Solutions Ltd. – CSC/PERCH NMR Software Course, Turku 16.-17.5.2006

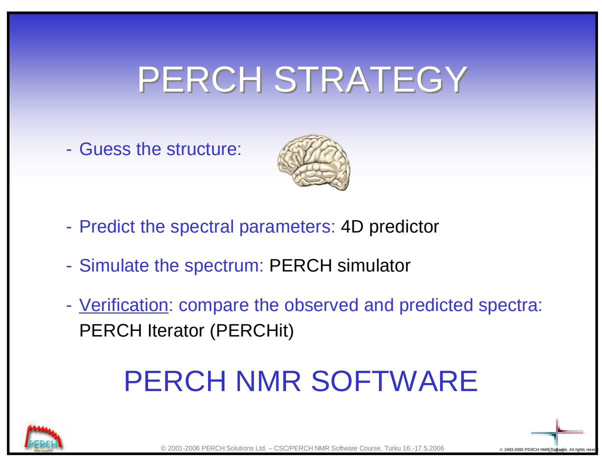

Problem
Observed Spectrum
Calculated Spectrum
No Overlap
$\frac{\partial I_n}{\partial P_i} \approx 0$
I_n
• Poor guess, the iteration diverges
© 2001-2006 PERCH Solutions Ltd. – CSC/PERCH NMR Software Course, Turku 16.-17.5.2006


Solution
Observed Spectrum
Calculated Spectrum
Overlap
$\frac{\partial I_n}{\partial P_i} \neq 0$
I_n
• Lower resolution, the iteration converges
© 2001-2006 PERCH Solutions Ltd. – CSC/PERCH NMR Software Course, Turku 16.-17.5.2006


Integral Transforms (IT)
Integral Transforms
A-Shaped Functions
SPAN
Spectrum
• All features smaller than SPAN are hidden.
© 2001-2006 PERCH Solutions Ltd. – CSC/PERCH NMR Software Course, Turku 16.-17.5.2006


Iterative process based on integral transform spectra
0.2 Hz
Iteration
Broadening 20 Hz
© 2001-2006 PERCH Solutions Ltd. – CSC/PERCH NMR Software Course, Turku 16.-17.5.2006


Problem
What happens with parameters << SPAN?
$\frac{\partial I_n}{\partial P_i} \approx 0$
Large Correction: Divergence!
Solution
Principle component analysis (PCA):
Only parameters having a significant effect on the spectrum are iterated (Threshold).
© 2001-2006 PERCH Solutions Ltd. – CSC/PERCH NMR Software Course, Turku 16.-17.5.2006


PERCH STRATEGY
- Guess the structure:
- Predict the spectral parameters: 4D predictor
- Simulate the spectrum: PERCH simulator
- Verification: compare the observed and predicted spectra: PERCH Iterator (PERCHit)
PERCH NMR SOFTWARE
© 2001-2006 PERCH Solutions Ltd. – CSC/PERCH NMR Software Course, Turku 16.-17.5.2006

Iterative Spectral Analysis

- Predict spectral parameters
- Simulate spectrum
- Compare to experimental spectrum
- Refine spectral parameters to match experimental data
- Evaluate quality of fit and difference between predicted and actual spectral parameters

Iterate

Why Computational Spectral Analysis?

- Structure verification
- More accurate spectral parameters
- Understand second-order spectra
- Analyze large spin-systems
- Can be automated

How can we use this?

Simulator-Iterator

- Up to 50 spin-systems (up to 50 spin-particles/system, overall 100 spin-particles)
- Up to spin 9/2
- Symmetric pairs and X-approximation for spin-1/2 particle
- Simulation of Cholesterol takes about 30 s (incl. line-shape, 450Mhz P2)

Spin System Notation

? AB ? AX ? A_2X ?

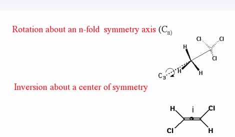
? ABC ABX ? ?

? ABMX AA'BB'XX' ?

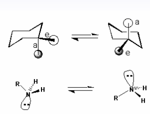
- Close chemical shifts → Letters close in the alphabet

Chemical & Magnetic Equivalence

Symmetry

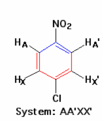


Rapid Exchange



- Same chemical environment
- Same resonance frequency → Chemically AND magnetically equivalent
- Same spin-spin interactions

Magnetic Equivalence



	Chem. shift eq.	Magnetic eq.
H_A, H_A'	Yes	No
H_A, H_X	No	No
H_X, H_X'	Yes	No

- Same resonance frequency
- Different spin-spin interactions → Chemically equivalent BUT NOT magnetically!

Multiplicity

	n	Chem. Eq.	Mag. Eq.	Notation
A	1	-	-	1x1
A2	2	Yes	Yes	1x2
A3	3	Yes	Yes	1x3
2A3	6	Yes	Yes	2x3
AA'	2	Yes	No	2x1

Scalar & Dipolar Couplings

Scalar couplings
- Through bonds
- Field independent
- Isotropic
- Multiplicity
- Sign

Dipolar couplings
- Through space
- Field independent
- Anisotropic
- Usually middles out in solution

PERCH's Iteration Modes

- Assignment (LAOCOON)
- Integral Transforms
- Total-Line-Shape Fitting
- Peak-Top Fitting

Why Automation?

- Increasing efficiency in data acquisition
  - Increasing amount of spectra to be analyzed
- Manual verification is time-consuming and requires expert level personnel
- Combinatorial chemistry