# *Shake-and-Bake:*
# Applications and Advances


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| | | |
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# Outline of Talk

SnB

- *Shake-and-Bake*
  - The Minimal Function
- *SnB*
  - Results
- *SnB* v2.0
  - Rationale
  - Results
- Summary

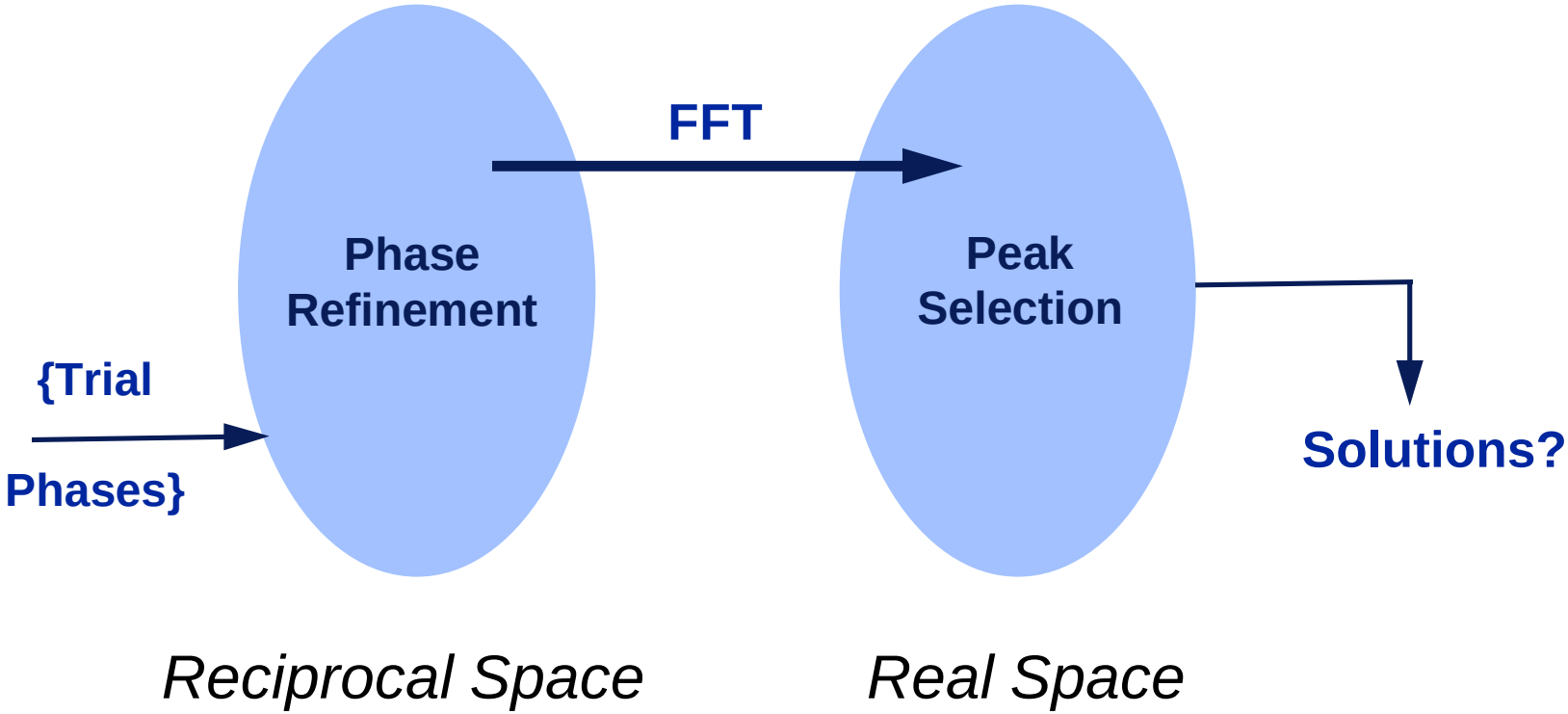
# Direct Methods

SnB

- ***Direct Methods*** **use probabilistic theories to exploit linear relationships among phases.**
- **Resolution of 1.2Å or better.**
- **Routinely applied to structures with 150 or fewer atoms.**
- **Standard packages:**
  - **SHELX**
  - **teXsan**
  - **SIR92/96**

# Conventional Direct Methods

SnB



# *Shake-and-Bake*

# The Minimal Function

SnB

$$R = \frac{\sum_T W_T \left(\cos\phi_T - est_T\right)^2}{\sum_T W_T}$$

Triple: $\phi_T = \phi_h + \phi_k + \phi_{-h-k}$

$$W_T = \left(\frac{2}{N^{1/2}}\right) \left| E_h E_k E_{-h-k} \right|$$

$est_T$ is the known expected value of $\cos\phi_T$

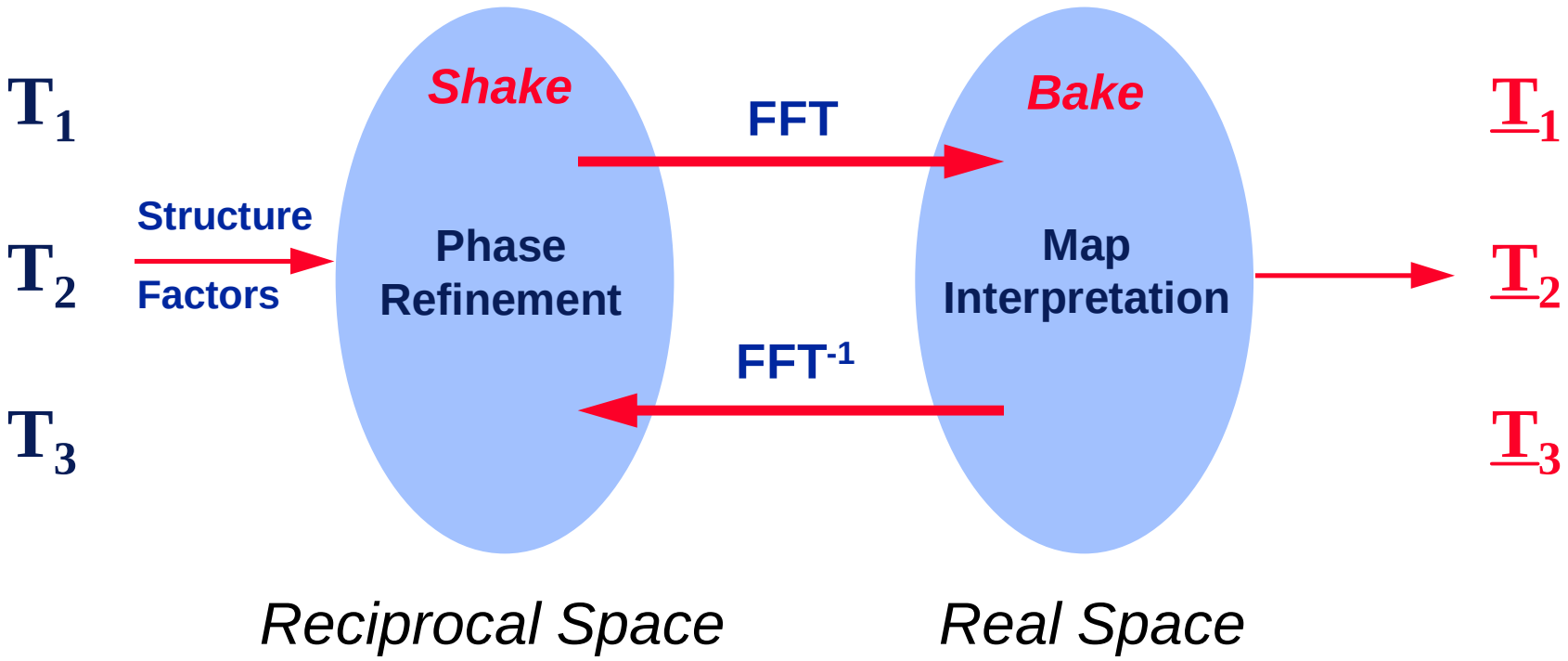
# *Shake-and-Bake*

SnB

- **Direct Methods Optimization Technique**
- **Multiple Random-Atom Trial Structures**
- **Real/Reciprocal Space Cycling**
- **Phase Refinement Techniques:**
  - **Parameter Shift**
  - **Tangent Formula**
- **Minimal Function as FOM**

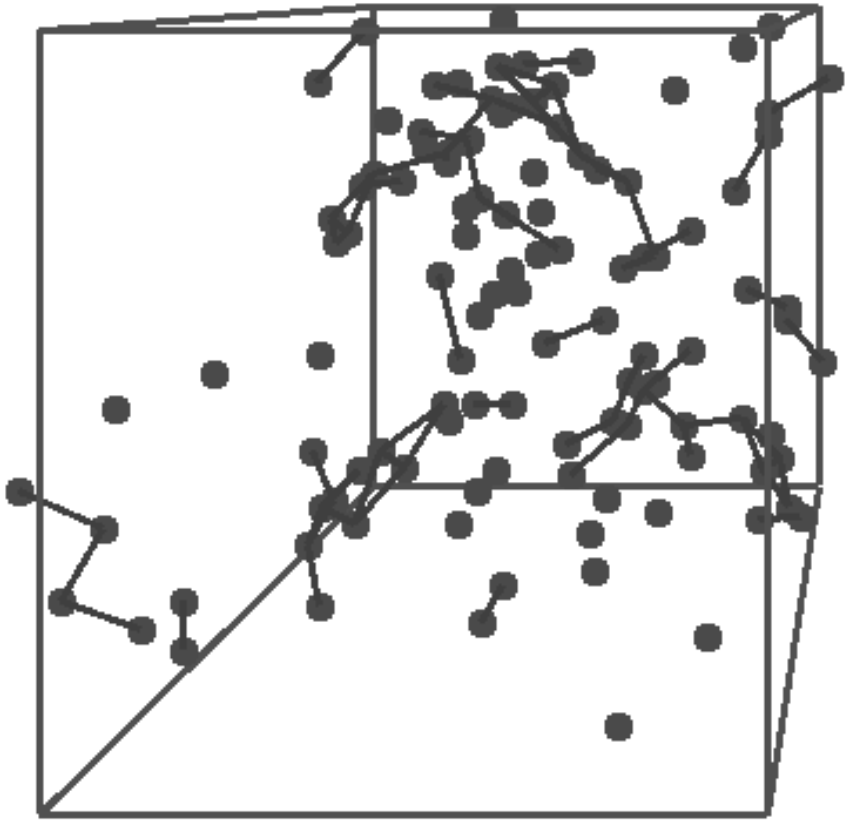
# *Shake-and-Bake*

SnB



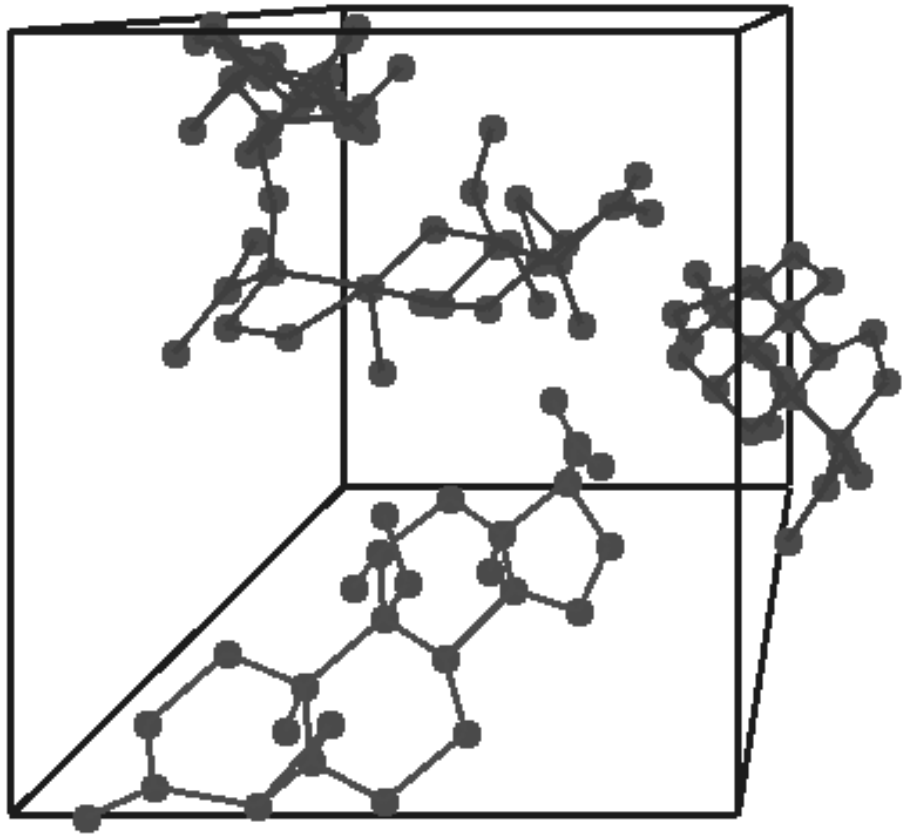
# *SnB*: Random Start

SnB

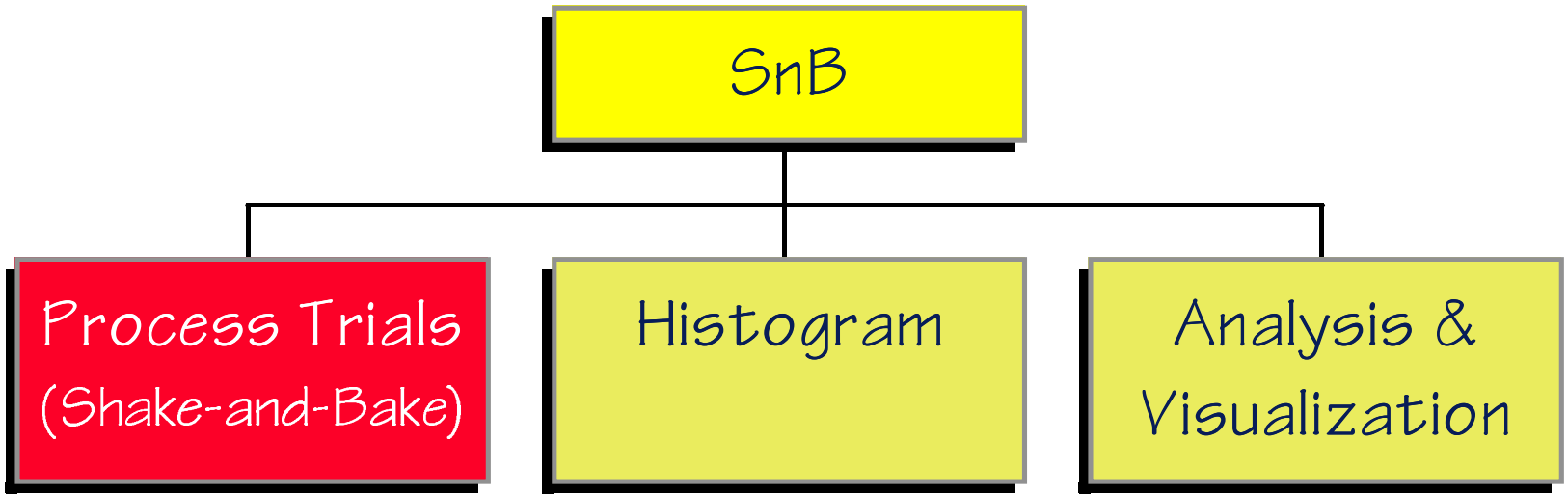


# *SnB*: Final Structure

SnB

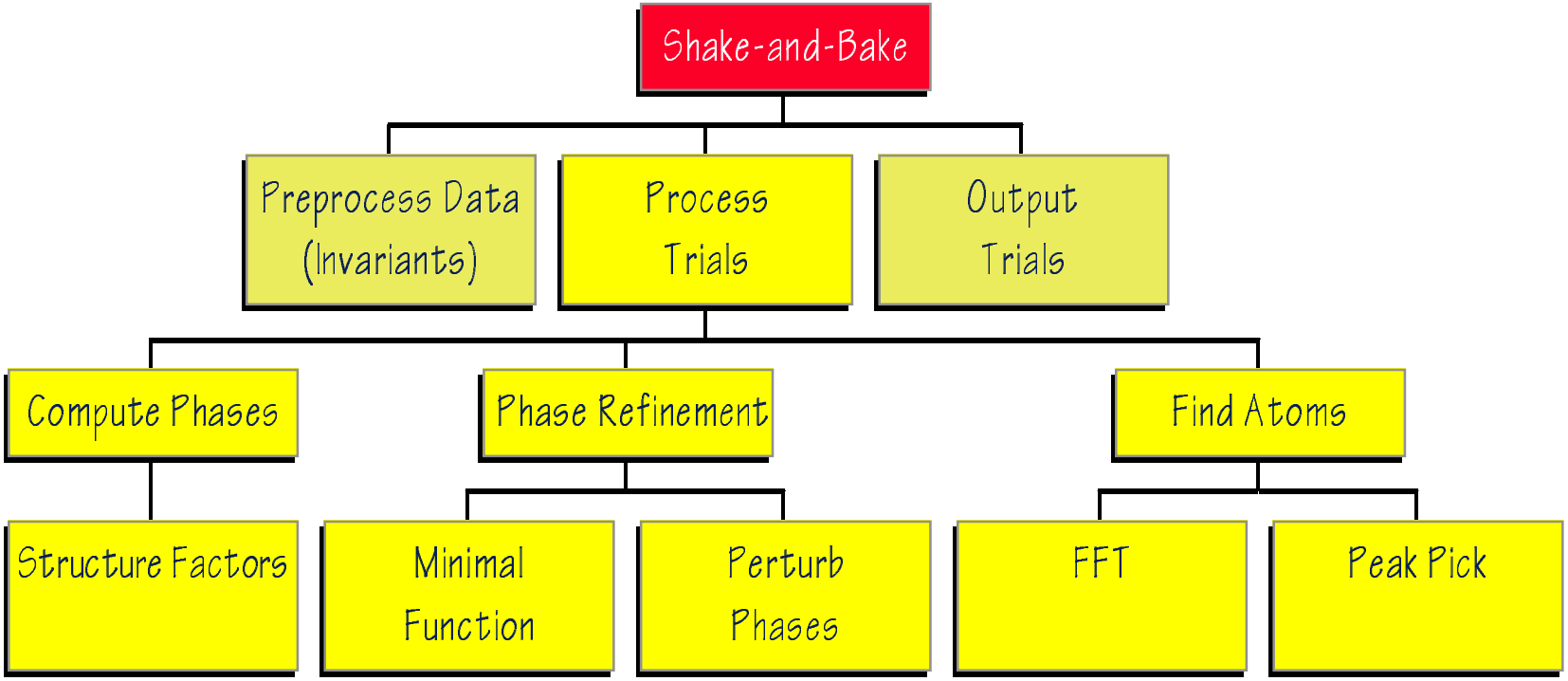


# Structure of *SnB*



# Shake-and-Bake

SnB



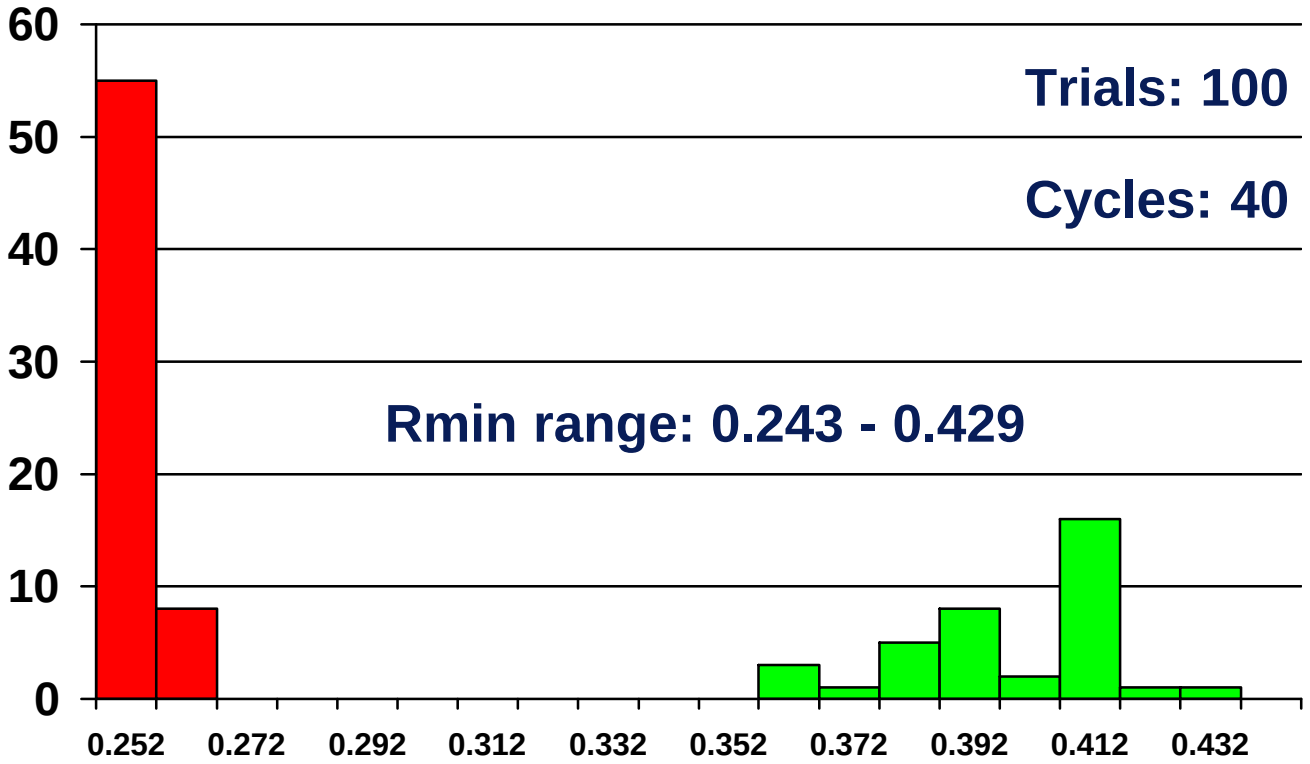
# *SnB* Parameters

| | Default | Ph8755 | ToxII |
|---|---|---|---|
| Atoms (asu) | *n* | 74 | 508 |
| Phases | 8*n* - 10*n* | 740 | 5,000 |
| Triples | 70*n* - 100*n* | 7,400 | 50,000 |
| Cycles (PS) | *n*/2 | 40 | 255 |
| Peaks recycled | 0.8*n* - *n* | 74 | 400 |
| E-Fourier Steps | 2 | 2 | 5 |

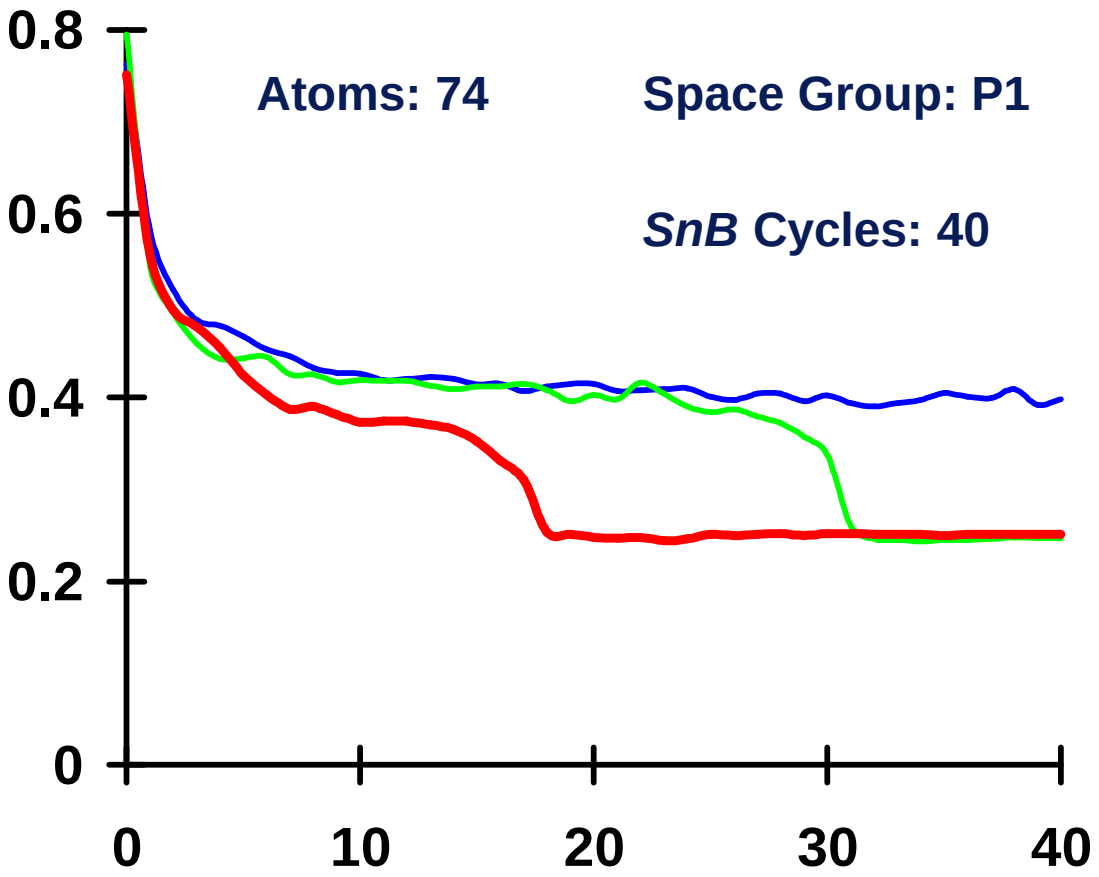
# Ph8755: *SnB* Histogram

Atoms: 74
Space Group: P1

Phases: 740
Triples: 7,400



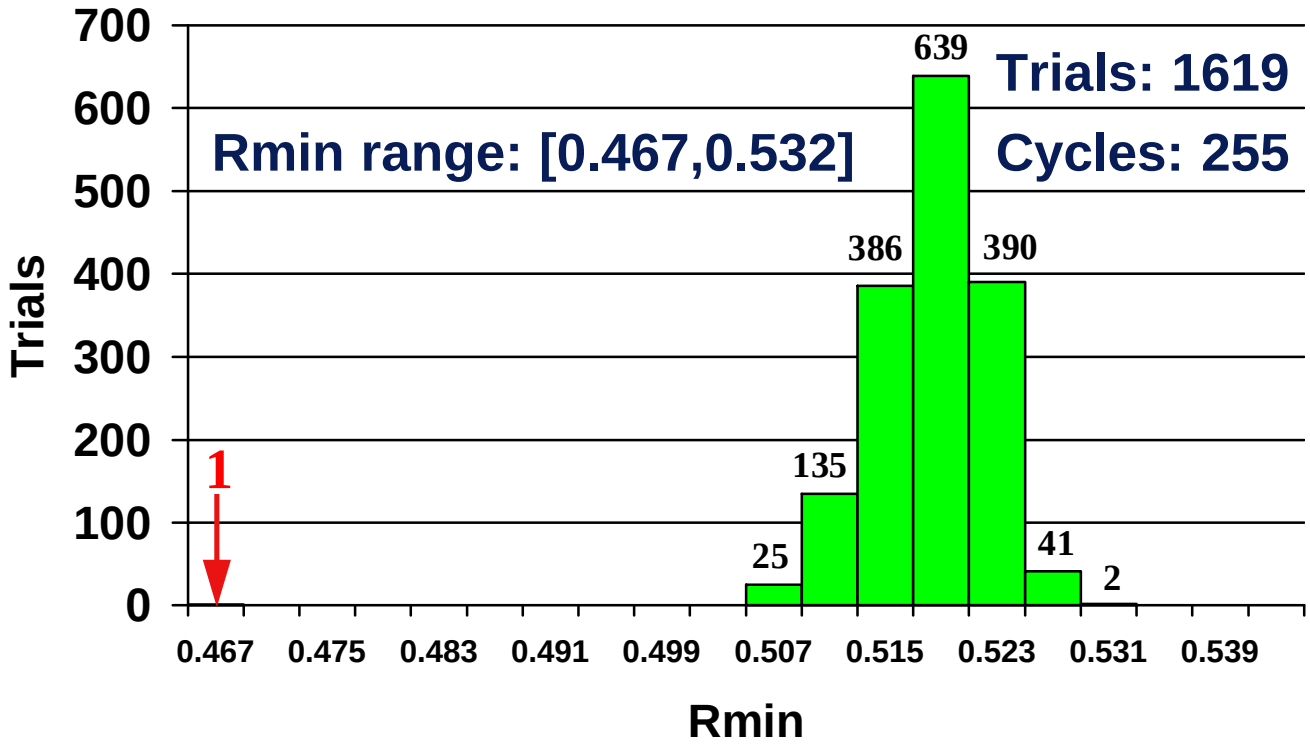
# Ph8755: Trace of *SnB* Solution



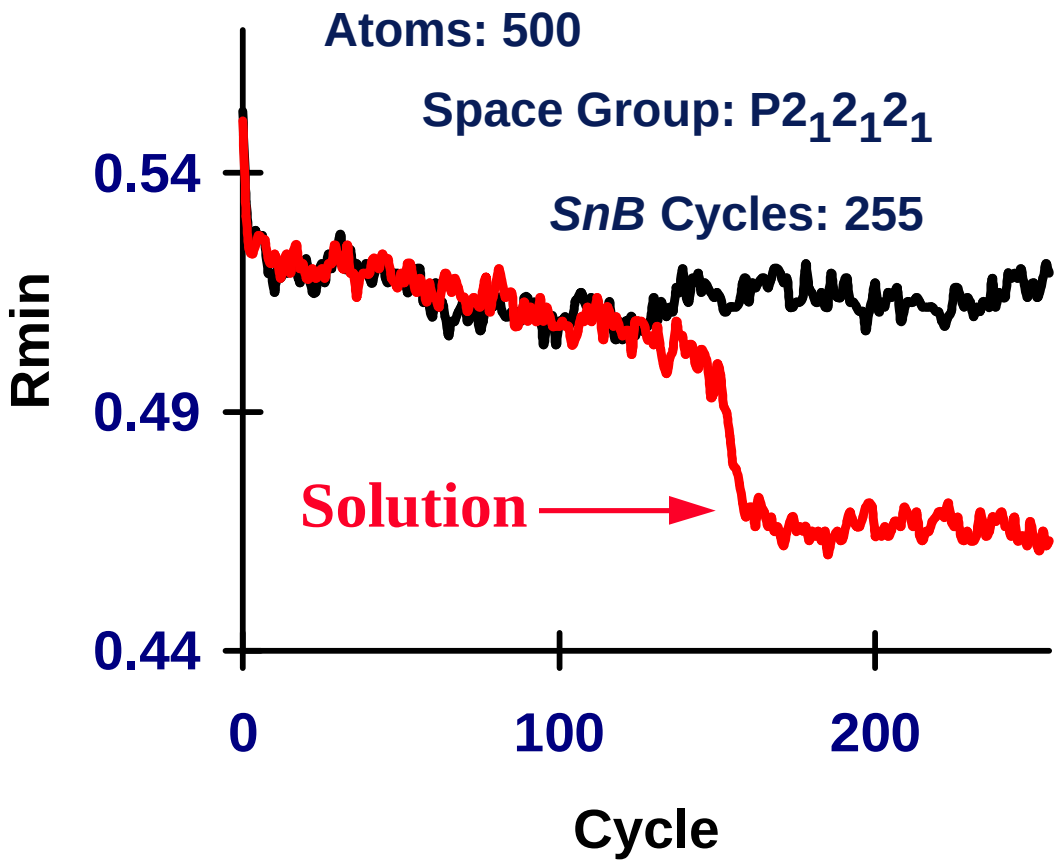
# ToxII: *SnB* Histogram

Atoms: 500
Space Group: $P2_12_12_1$

Phases: 5,000
Triples: 50,000

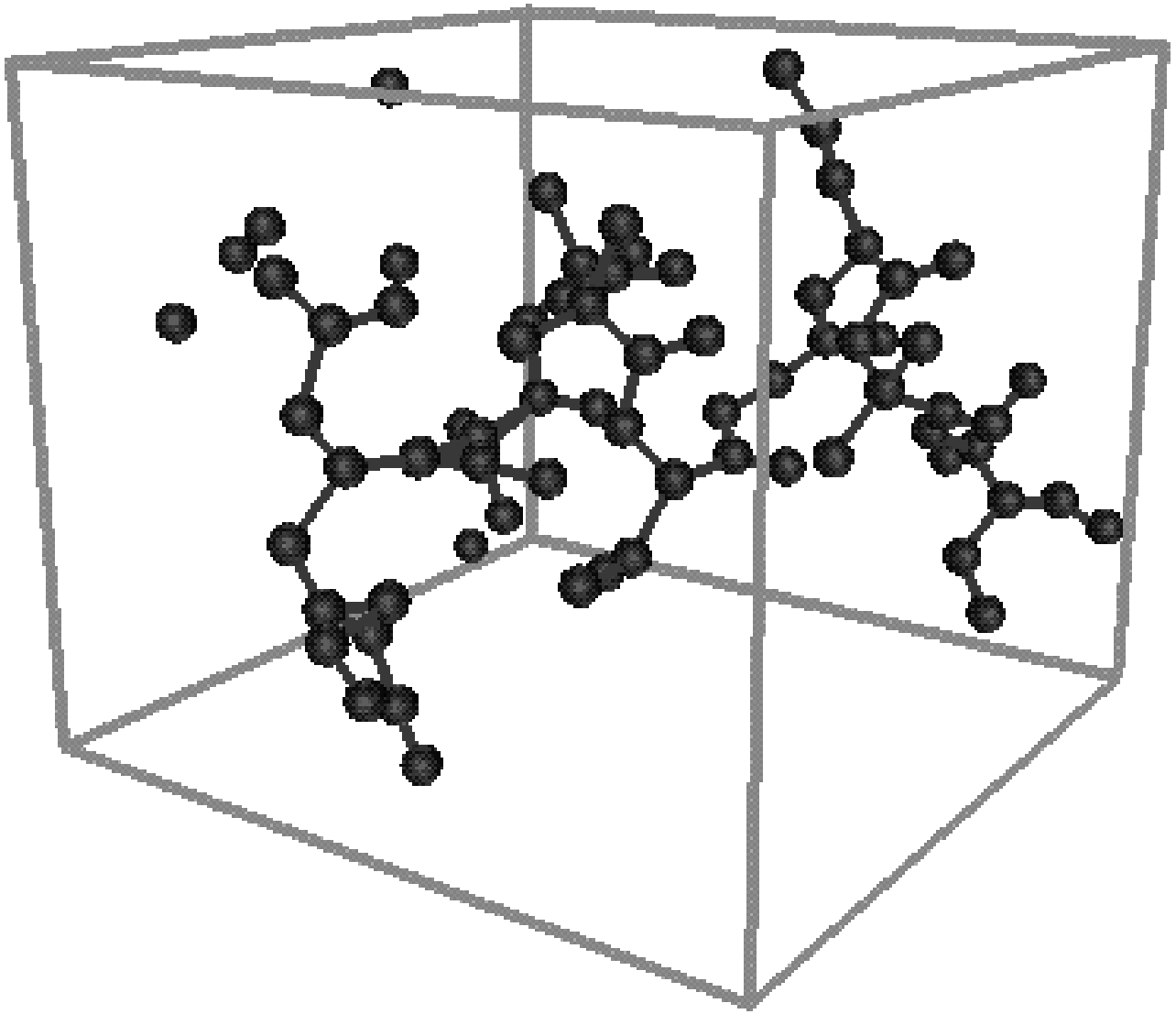


# Tox II: Trace of *SnB* Solution



# Visualization in *SnB* (Ph8755)

## *Geomview:* Geometry Center, U. Minn.



# Some *SnB* Applications

| Structure | Location | Atoms | Sp Grp | Res |
|---|---|---|---|---|
| Vancomycin | Penn | 258 | $P4_32_12$ | 0.9Å |
| I4 Peptide | HWI | 289 | I4 | 1.1 |
| Microlide | France | 296 | $P2_1$ | 1.1 |
| Gramicidin A | HWI | 317 | $P2_12_12_1$ | 0.86 |
| Er-1 pheromone | UCLA | 328 | C2 | 1.0 |
| Crambin | HWI | ~400 | $P2_1$ | 0.83 |
| Alpha-1 peptide | OCI/U. of T. | 471 | P1 | 0.92 |
| Rubredoxin | HWI | 497 | $P2_1$ | 1.0 |
| Scorpion Toxin II | HWI | 624 | $P2_12_12_1$ | 0.96 |

# Factors Determining Success Rate

SnB

- **Data quality**
- **Resolution**
- **Complexity and connectivity of structure**
- **Space group**
- ***Presence of heavy atoms***

# An Interesting I4 Structure

SnB

- **Structure:**
  - Peptide with 10 Sulfurs
  - 289 nonH atoms total
  - 1.1Å resolution data
- **Bugs: Special Positions & Refinement**
- **Results (*SnB* 2.0)**
  - PS: 53% success rate
  - PS/Rest: 44% success rate
  - Tan: 25% success rate

# Extending Resolution: the I4 Structure

SnB

- **Truncate to 1.2Å - 1.5Å**
- **Solutions at all resolutions**
  - **1.2Å:** Standard bimodal distribution
  - **1.3Å:** Standard bimodal distribution
  - **1.4Å:** Good, but some mixing of solutions and nonsolutions
  - **1.5Å:** Solutions (~50 deg. phase error) but *not* recognizable by FOM
- **Recognize low-resolution solutions??**

# *SnB* 2.0: Rationale

- **Improve running time**
  - Build from ground up
- **Provide additional features**
  - Inverse Fourier
  - Density modification
  - Grid size
  - “Twice Baking”
  - Peaks at special positions

# *SnB* v2.0 Parameters/Proteins

| *Structure* | *Atoms (n)* | *Heavy Atoms* | *Phases* | *Cycles* | *Max Succ Rate* |
|---|---|---|---|---|---|
| Vancomycin | 202 | *Cl* 8 | 2000 | 200 | 0.8% |
| I4 Peptide | 248 | S10 | 1900 | 250 | 53.0 |
| Gramicidin A | 272 | - | 3000 | 275 | 1.1 |
| Crambin | 327 | S6 | 3000 | 300 | 4.8 |
| Rubredoxin | 395 | FeS6 | 4000 | 400 | 6.2 |
| Scorpion Toxin II | 508 | S8 | 5000 | 500 | 1.4 |

**Note: *n* = independent protein atoms**

# *SnB* 2.0: Varying Peaks

| *Structure* | 50 | 100 | 200 | 300 | 400 |
|---|---|---|---|---|---|
| Vancomycin | 0.4% | 0.6% | 0.2% | --- | --- |
| I4 Peptide | 53.0 | 52.0 | 45.0 | --- | --- |
| Gramicidin A | 0.0 | 0.3 | 1.1 | 0.7% | --- |
| Crambin | 4.3 | 4.8 | 3.3 | 3.4 | --- |
| Rubredoxin | 5.7 | 6.2 | 5.4 | 3.9 | 3.4% |
| Scorpion Toxin II | --- | 1.0 | 1.4 | 0.4 | 0.1 |

# *SnB* 2.0: Varying Cycles

**Success Rates while varying *SnB* Phase Refinement Cycles**

| Structure | 0.25*n* | 0.5*n* | 0.75*n* | *n* | 1.25*n* | 1.5*n* |
|---|---|---|---|---|---|---|
| Vancomycin | 0.1% | 0.4% | 0.4% | 0.6% | 0.7% | - |
| I4 Peptide | 27.0 | 40.0 | 48.0 | 53.0 | - | - |
| Gramicidin A | 0.0 | 0.4 | 0.6 | 0.9 | 1.2 | 2.0% |
| Crambin | 3.1 | 4.1 | 4.6 | 4.8 | - | - |
| Rubredoxin | 4.6 | 5.5 | 5.9 | 6.0 | - | - |
| Scorpion Toxin II | 0.05 | 0.5 | 1.0 | 1.4 | - | - |

# *SnB* 2.0: Phase Refinement

| *Structure* | *Peaks* | *PS Standard* | *PS Restricted* | *Tangent* |
|---|---|---|---|---|
| Vancomycin | 100 | 0.6% | 0.4% | 0.3% |
| I4 Peptide | 50 | 53.0 | 44.0 | 25.0 |
| Gramicidin A | 200 | 1.1 | 0.5 | 0.0 |
| Crambin | 100 | 4.8 | 3.7 | 2.2 |
| Rubredoxin | 150 | 6.0 | 5.2 | 4.0 |
| Scorpion Toxin II | 200 | 1.4 | 1.0 | 0.7 |

# *SnB* 2.0 Parameters (>1.1Å)

- **Peaks**
  - **0.4*n* if "heavy" atoms present**
  - **0.8*n* if all *C,N,O***
- **Phase Refinement**
  - ***Unrestricted* Parameter Shift**
- **Cycles**
  - ***n*/2 if *n*<400 and "heavy" atoms present**
  - ***n* otherwise**

# *SnB* 2.0: Timings
## (SGI R10000 Workstation)

| *Structure* | *non-H Atoms* | *Space Group* | *n/2 Cycles* | *Trials/ Day* | *Solns/ Day* |
|---|---|---|---|---|---|
| Vancomycin | 258 | $P4_32_12$ | 100 | 391 | 1.5 |
| I4 Peptide | 289 | I4 | 125 | 274 | 110 |
| Gramicidin A | 317 | $P2_12_12_1$ | 135 | 572 | 2 |
| Crambin | ~400 | $P2_1$ | 150 | 1029 | 42 |
| Rubredoxin | 497 | $P2_1$ | 200 | 294 | 16 |
| Scorpion Toxin II | 624 | $P2_12_12_1$ | 250 | 109 | 0.5 |

**Note: For each structure, optimum no. of peaks used.**

# Computing Platforms

SnB

- Unix Workstations
  - SGI, Sun, DEC/Alpha
  - Wintel/Linux
- Parallel Computers
  - Cray T3D/E, TMC CM-5, IBM SP2
  - SGI Origin 2000
  - HP-Convex Exemplar
- Cray C90

# Summary

SnB

- ***Shake-and-Bake:* Dual-Space Direct Methods**
- **Targeted at 100-800 atom structures**
- ***SnB* version 2.0**
  - Optimized code with Inverse FFT
  - Additional Density Modification Options
  - Improved Fourier Recycling: “Twice Baking”
  - I/O: |E| calculation and visualization interface
  - (SIR/SAS/MAD Invariants with estimated values)
- **http://www.hwi.buffalo.edu/SnB/**


SnB