I  NMR screening protocol
Story of the samples after they leave the third floor!

II  Keeping track of the entire process starting with NC5 samples
Biography of the selected targets

Swapna Gurla / G V T Swapna
NMR spectroscopist
NMR screening of the NC5 samples for structure determination
Using NMR Spectroscopy

Step I
Fill in the table generated by Karishma with info of size of the proteins & aggregation screening results. Need 0.4 – 1.2mM conc.

Acquire 2D NH HSQC, 1D HetNOE, 2D HetNOE, 1D T1 array, and 1D T1rho array, at 20 deg C on V500

Decide if folded? Good S/N? Stable & how long? Enough pks?

Folded. Good/Excellent HSQC. Good S/N. Protein very stable at room temp. Nearly complete Peak-count.
Create jpg file of HSQC spectrum. Run high-resoln Non-CT 2D CH-HSQC
Upload the NH-HSQC, 1D-2D HetNOE, T1, T1rho and 2D CH-HSQC raw data into SPINS. Check SPINE upload
Perform Competition Analysis, check aggregation screening results, tauC estimation. Update “NMR Progress table” in SPINE

Promising: Good HSQC but sample is precipitating, or very low sample concentration, or addnl minor peaks or OK at one buffer
Request Button testing for best Conditions of Buffer and temp. Get new NC5 samples. Treat like a new target. Begin Step I

Swapna stores NC5 sample Until ready for shipment.

Unfolded? Not enough peaks because of protein size? Try Higher field if there is hope. If not, OUTBOX

Check who can be assigned the Target. Suggest the researcher. Okay CN label at best NC5 buffer

Mike sends an e-mail to Rong asking for CN label. E-mail to the researcher who Has been assigned the target

Poor: Very few peaks than Expected. Very low S/N although sample conc. is good. Aggregating. Unusually long Tauc. ABANDONED

Rong’s grp enters PST ID for each NMR sample into SPINS. SPINS creates the target PST As category 0 in SPINE NMR progress summary

E-mail to the researcher who Has been assigned the target
NMR screening of the NC samples for structure determination
Using NMR Spectroscopy

**Step II**

- Acquire 2D NH HSQC, 1D HetNOE, 2D HetNOE, 1D T1 array, and 1D T1rho array, at 20 deg C on V500

- Decide if folded?
  - Good S/N?
  - Stable & how long?
  - Enough pks?

- Unfolded/Poor Not enough peaks because of protein size? Try Higher field, lower temp. If not, ABANDON SHIPPING. Discuss with Guy.

- Folded. Good/Excellent HSQC. Good S/N. Protein very stable at room temp. Nearly complete Peak-count.
  - Create jpg file of HSQC spectrum. Overlay with NC5 spectrum.
  - Upload the NH-HSQC, 1D-2D HetNOE, T1, T1rho and 2D CH-HSQC raw data into SPINS. Check SPINE upload
  - Okay for shipping to the assigned Researcher. Update The status in “NMR progress table”
  - Sample SHIPPED. Mike takes over tracking progress in structure determination Until it goes into PDB

- Good HSQC but sample is precipitating, or very low sample concentration, or addnl minor peaks or OK at one buffer
  - Request Button testing for best Conditions of Buffer and temp. Get new NC5 samples. Treat like a new target. Begin **Step I** or target is shelved or ABANDONED with justification reported to Guy and Mike

- Swapna gives NC5 sample And NC sample to Rong for shipping. NC5, NC to researcher 200ul NC5 to J.Pretegard
Archiving NMR data and keeping track of the “Progress”

NESG NMR Progress Summary
Sept. 22, 2005

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<th>NESG Id.</th>
<th># of Residues</th>
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<th>Comments</th>
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Status Class A:
Good/Excellent HSQC

<table>
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<th>Status Class A: Good/Excellent HSQC</th>
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<td>SrR68  102  Good  Clean  -  -  A</td>
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<td>SrR73  115  Good  Clean  Very low sample  -  A</td>
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Index:
A: Good/Excellent HSQC
B: Button test
C: assigned and ordered CN sample
D: CN sample shipped
E: In data collection
F: In Refinement
G: In PDB
H: Abandoned
**NMR Progress Summary**

This page shows a progress summary for NMR targets. There are currently 49 records in the database meeting the search criteria.

### NESG NMR Target Distribution

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Initially sent to PNNL. The tauC is too big by 1.5x and their are less than 59 strong peaks, along with another 29 or so weak peaks. The weak peaks have larger linewidths and don't show up at all in the 3D HNCA/CO suggesting that they belong to a larger aggregate. The aggregation screening in SPiNE shows a higher molecular weight species in some of the

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5
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Add a target to the list:  [ ] Submit

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Database Home

Some Authors
Last modified 31-09-45