MLPCN Frequently Asked Questions

Production U54 FAQs

A. Review main differences between Pilot Phase and Production Phase

The production phase of the Molecular Libraries Probe Production Center Network (MLPCN) will differ from the pilot phase (MLSCN) in several strategic ways based on knowledge and on experience gained during the pilot phase.

- In contrast to the ten similarly structured centers of the pilot phase, the MLPCN will be composed of three different types of centers working together as a network to provide improved handling of the diverse classes of investigator submitted assays as well as enhanced chemistry support for the large number of active compounds identified by the screening centers;
- With the advent of Specialized Chemistry Centers expanding program chemistry resources, there is an increased emphasis on network communication and interaction;
- In addition to providing a probe development resource for investigator submitted assays as a service to the community, each Center will have the option of initiating center-driven research in support of MLP goals;
- Each screening center will be expected to have an assay development/assay implementation function to permit development of investigator submitted assays that require minor modification before screening;
- The first step for all centers is the creation of a Compound Probe Development Plan (CPDP) by the project team composed of center staff, the assay provider and a NIH Network Science Officer assigned to the assay project. The CPDP outlines the projected probe development path for the specific assay, assigns tasks to each member of the project team and predicts appropriate milestones and timelines;
- Each screening center will be expected to provide an active outreach program to increase interest of the biomedical community in submitting assays to the MLPCN and provide support to aid an assay submitter in preparing a competitive X01 or R03 Assay Solicitation application;
- Each screening center should provide support to assay providers for scaling up production of reagents necessary for performing HTS and secondary assays;
- Each screening center should expect to run the primary and all secondary screening assays, including one or more alternative screens, as defined in the Chemical Probe Development Plan (CPDP).

B. Frequently Asked Questions

#1 (all centers)

Q: In terms of meeting the user-driven needs, are we allowed to request more than 70% of the direct cost cap per year for that purpose?

A: The user-driven component has no limit up to the direct cost cap for the center type.

#2 (Specialized Screening Centers)

Q: According to the new RFA, are the specialized screening centers still allowed to do cheap and fast post-HTS SAR evaluations of commercially available compounds in order to provide

the collaborating Chemistry Centers with better starting points for structure-activity optimization?
A: Ideally the screening center has matched with a chemistry center before starting the primary screen; certainly no later than at the end of the primary screen. We recognize that the type of structures found in the initial hits may be a factor in selecting the appropriate chemistry center. The chemistry centers are better prepared to study the structures of hits from the primary screen and select the best commercially available compounds to be screened next. The collaboration between screening and chemistry centers will be close and continue to the end of the probe project. The screening center will provide all secondary screening including counter screens and work with the chemistry center in evaluating and interpreting data as they jointly work to identify the optimum probe.

#3 (all centers)
Q: Is it anticipated that several (e.g. 5-6) independent multi-year projects could be proposed per Center? How many projects per Center seems reasonable to NIH?
A: Applicants can propose more than one multi-year proposal for their center-driven component.

1. Successful applications will be those with a strong user-defined component. The center-driven component could be important when the user-defined portion of two applications is closely scored and only one can be funded.
2. Funding for the center-driven component can be as large as 30% of the direct costs for the FOA ($3M for comprehensive centers). However, the center-driven budget may be adjusted down depending on reviewer's comments, program staff's assessment of project costs and the availability of funds. Such adjustments in the center-driven component are not moved into the user-defined component and are lost to the center. It is recommended to first make sure your budget is adequate to meet all user-defined center goals and then apply the remaining funds to the center-driven aims.
3. Proposed center-driven projects that result in assays that are the same or similar to assays provided by the scientific community to the MLSCN will not be considered.

#4 (all centers)
Q: Does the $500K cap apply to center-driven proposals? For example if a center-driven proposal is for new technology or instrumentation development that requires the purchase of instrumentation, does the cap apply?
A: No. There is no cap on instrumentation needed for center-driven proposals.

#5 (all centers)
Q: Will the reviewers of applications for the U54 production phase study section have access to the X02 summary statements?
A: The reviewers will not have access to the X02 summary statements as the X02 is a different grant mechanism than the U54 as well as some applicants were asked to submit proposals for a different center type than their X02.

#6 (screening centers)
Q: Why does a center require an "assay development core" and what is its purpose?
A: Centers will need to plan on providing some assay development to address the range of "HTS-ready" assays that will be assigned. The assay development capability should compliment assay implementation and only provide minimal assay modification to improve and adapt the existing assay to run at the center. The assay development capability will also be important in preparing and running secondary screens including counter screens to define probe specificity for a target. The term "assay development" in the RFA does not mean that we expect centers to generate their own set of assays. If there is excess assay

development capacity, the centers are certainly encouraged to develop assays that may be submitted to the MLPCN through the X01/R03 Assay Recruitment RFAs.

#7 (all centers)
Q: On page 22 of the RFA, it says "The overview must contain a Summary Table with all the functional cores in the Center." Are you looking for more of a flowchart showing the interrelatedness of the cores?
A: The table format will provide the reviewers with a clear overview of the pipeline and how the cores within a center work together smoothly to maintain the pipeline. The table provides the center with a simple way to convey to the reviewers that the process is well thought out and integrated. The table should include the capabilities and responsibilities of each core, the number of staff supporting the core and how it is managed. The Specialized Screening and Chemistry Centers have fewer cores but they should also try to apply the table format to describe the separate steps in their processes.

Frequently Asked Questions:
Pre-application for The Molecular Libraries Probe Production Centers Network (MLPCN)

Program Announcement: PAR-07-368 (X02)

This website will consolidate all the questions we get and our responses. Please continue to consult this website as you develop your application.

The X02 mechanism

This is a new pre-application mechanism that allows peer-review but does not result in an award. It is used by the NIH for dealing with challenges involving (1) large number of applications, (2) complexity of application review, among others. These pre-applications require less than 25 pages generally, the CRC X02 has a 20 page limit. Please refer to the X02 notice for details on how the peer-review will be undertaken and the process that would follow resulting in an invitation to submit a full application to the CRC FOA (funding opportunity announcement).

The U-54 co-operative agreement

U54's are "Specialized Center--Cooperative Agreements". Their purpose is to support any part of the full range of research and development from very basic to clinical; may involve ancillary supportive activities such as protracted patient care necessary to the primary research or R&D effort. The spectrum of activities comprises a multidisciplinary attack on a specific disease entity or biomedical problem area. These differ from program project in that they are usually developed in response to an announcement of the programmatic needs of an Institute or Division and subsequently receive continuous attention from its staff. Centers may also serve as regional or national resources for special research purposes, with funding component staff helping to identify appropriate priority needs.

The U54 is a cooperative agreement award mechanism. In the cooperative agreement mechanism, the Principal Investigator retains the primary responsibility and dominant role for planning, directing, and executing the proposed project, with NIH staff being substantially involved as a partner with the Principal Investigator. The details of terms and conditions for such cooperative agreements will be present in the RFA (not X02 Sample co-operative agreements that the applicant may wish to read to get a general idea can be found in the RFAs for the MLSCN (see section "Cooperative Agreement Terms and Conditions of Award") as well as NCBC.

General help about NIH grants

A general tutorial on grants from NIH can be found at this website: http://www.niaid.nih.gov/ncn/grants/default.htm. However, please read the disclaimers found there and notice that it is primarily focused on R01 grants. There are other websites with helpful advice available at the NIH though scattered in multiple places. The main NIH website with detailed information about grants is http://grants.nih.gov/grants/index.cfm. An introduction to electronic applications (X02) can be found here http://era.nih.gov/ElectronicReceipt/index.htm.

Description of Molecular Libraries Centers

Three separate types of Centers are requested in this RFA. The three types of Centers are defined below. Each Center type will provide a specific function to the network and will be required to contain specific capabilities. The general steps required to take an assay through the discovery process to identification of a probe are defined in the figure below. An overview of the different functions provided by each type of Center is also provided in the figure below.

I. Comprehensive Screening Centers: The Comprehensive Centers will provide rapid screening on a broad diversity of assays and detection platforms (e.g., enzymes/proteases, G-protein coupled receptors, kinases, cytotoxicity, protein-protein interactions, protein misfolding/degradation, high-content screening, transcription/expression, etc.). These large, ultrahigh-throughput screening centers will contain all the necessary functions to take a project through the complete chemical probe development process from assay development to chemical synthesis of potent and selective probe compounds. The key functionalities or cores required by these centers are: assay development, assay adaptation/implementation, HTS, informatics and chemistry. A Comprehensive Center will be run under process management where production rate, program efficiency and costs will be carefully monitored by NIH program staff. Each center is expected to screen a library of 300K-500K compounds per assay and to process 25 or more assays per year. A Comprehensive Center will be responsible for all three operational stages in the probe generation pipeline:

- Hit identification: Provide capability for assay adaptation/implementation, HTS and informatics to accept assays into the center, modify and implement them for automated high-throughput screening; for screening the MLSMR library of compounds; and for confirming initial hits by re-screening. All quality control (QC), primary and reconfirmation data will be analyzed by the informatics core and immediately deposited into PubChem.

- Hit characterization: Fresh samples of initial hits will be selected (cherry-picked) by the MLSMR and sent to the centers for further characterization. The HTS core will run the samples at a series of concentrations to generate dose-response curves which are invaluable in identifying false positive hits. Working with the assay provider, valid hits will be characterized in an alternative (secondary) assay to that used in the primary screen. This provides additional verification that the hit is acting on the target of interest. Depending on the assay and target, the assay implementation and HTS cores will develop counter-screens to determine the selectivity and specificity of the hits for the target. The informatics and chemistry cores will analyze the data for correlations between compound structure and the observed biological activity (SAR analysis). All assay data on hit characterization will be promptly deposited into PubChem.

- Hit optimization: To optimize on the hit information, the assay implementation, informatics and chemistry cores work together through successive rounds of testing of new compounds to improve the potency and selectivity of the original hits. New compounds for testing in this phase may be purchased from commercial sources or obtained through chemical synthesis by the chemistry core of the center.

II. Specialized Screening Centers: Certain types of assays requiring multiple steps or specialized instrumentation (e.g., phenotypic imaging assays, multiplexing flow cytometry, ion channel assays) may not fit well in the comprehensive center format due to their special requirements and slower screening rates. These assays are critically important in the identification of protein function in cells and signaling pathways, yet pharmaceutical companies will not run these assays because of their low throughput and questionable disease relevance. However, assays of this type are windows to important biological phenomena and are an important objective of the MLP. Therefore, the MLP has introduced the concept of Specialized Screening Centers in the production phase. These centers will be smaller in scope, intended to focus on a specific type of assay or platform, and expected to complete a limited number of assays per year (about 5). These centers will have assay development, assay adaptation/implementation, screening and informatic functional cores. They will accept assays from the scientific community, as well as generate their own assays.

• Hit identification: The Specialized Centers will run the primary screen, confirmatory assays and analyze all the data before deposition into PubChem. The throughput of many of these assays will intentionally be relatively low because the emphasis of the specialized center's component of the MLPCN is not screening rate but the opportunity to include other medium and low throughput screening methods for difficult assays. Complete screening of the MLSMR library is expected, but this will occur over a longer time span than an assay campaign in the comprehensive centers.

• Hit characterization: Immediately following hit confirmation, fresh samples of initial hits will be selected (cherry-picked) by the MLSMR and sent to the Specialized Screening Center for further characterization. The center will run the samples at a series of concentrations to generate dose-response curves to identifying false positive hits. Working with the assay provider, valid hits will be characterized in an alternative assay to verify the compound is target specific. Depending on the assay and target, the center will develop counter-screens to determine the selectivity and specificity of the hits for the target.

• Hit optimization: At an early stage in hit characterization, the Specialized Screening Center will identify within the MLPCN a Specialized Chemistry Center or Comprehensive Screening Center capable of providing chemistry resources for hit-to-probe optimization. A hit-to-probe strategic plan will be developed by the two centers and the assay provider. The chemistry center will commit its cheminformatics and chemistry resources to advance potential hits to a final chemical probe. In this collaborative effort, the Specialized Screening Center will provide all follow-up assays on the compound analogs..

III. Specialized Chemistry Centers: The "hit-to-probe" optimization stage in the probe development process requires special tools and expertise, resources that are frequently in short supply in a screening center. Specialized Chemistry Centers will perform cheminformatics, synthetic medicinal chemistry and limited pharmacology to advance active compounds identified by the Specialized Screening Centers or, in some instances, in the Comprehensive Centers. It is essential that compounds identified as research tools for the investigation of particular targets and biological processes by the MLPCN are reliable and capable of providing valid information on the activity of their intended targets. The probe compounds produced by the centers will need to be usable by the research community for in vitro and/or in vivo studies. In most cases, compounds identified by initial screening ("hits") will not be ideal as research tools. It is likely that properties such as affinity, specificity, and solubility will need to be improved through the combined effort of the chemistry resources of the Specialized Chemistry Center and rescreening of new compounds by the Specialized Screening Center.

• Hit optimization: Probe leads identified from screens in the Specialized or the Comprehensive Screening Centers will require further optimization through chemistry. A joint effort will begin during the hit characterization stage between the screening center and the chemistry center for hit-to-probe optimization. This joint effort will match the rich chemistry resources of the Specialized Chemistry Center with the counter-screen and follow-up screens of the Screening Center in a close collaboration. A chemical probe development plan will be generated by the two centers and the assay provider where the chemistry center will commit its cheminformatics and chemistry resources to advance potential hits to a final chemical probe. In this collaborative effort, the screening center will provide all follow-up assays on the compound analogs. The chemistry center will need to provide sufficient synthetic chemistry to generate a library of structurally related compounds (~100-300) to identify compounds of improved affinity, specificity and solubility.

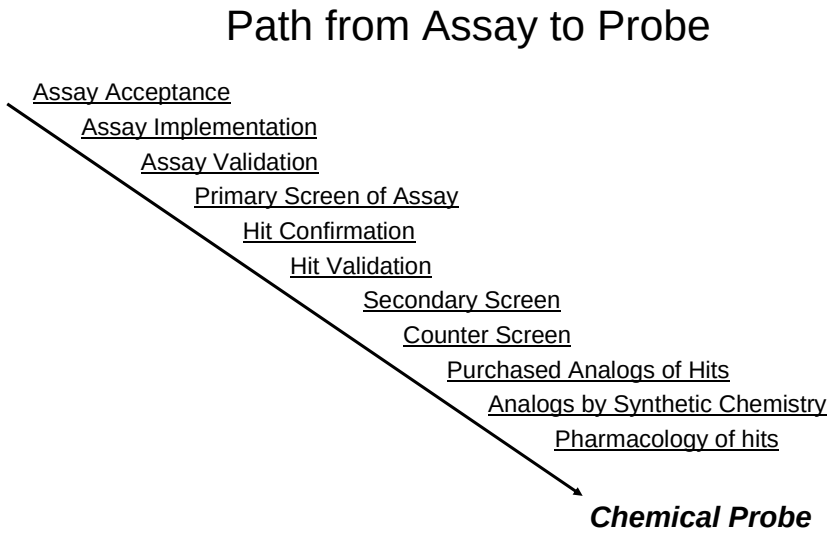
Chemical Probe Guidelines:

The minimum properties displayed by a compound to be defined as a chemical probe are:

1. Development of a new probe: must represent an improvement over the existing art.
2. Potency: <1 – 10uM; context dependent (varies with assay target & current state of the art; example: new compound provides similar potency but improved selectivity)
3. Solubility: sufficient solubility in relevant assay solvents.

4. Availability: accessible in amounts to allow advanced studies (15-20mgs)
5. SAR, mode of action, selectivity and toxicity are important compound information to have but not required.

Chemical Probe Development Path:



Center Infrastructure:

Pipeline: ASSAY PROBE

Center cores: Adaptation/implement[1] – - - HTS – - - - Informatics - - – Synth Chem.
Comprehensive Cntrs:
Special Screening Cntrs:
Special Chemistry Cntrs:

[1] See list of terms below.

Definition of Terms:

Compounds

Active Compound Series: structurally related compounds demonstrating similar biological activity.

Active Well: a position in a multiwell screening plate that demonstrates the desired response following a primary screening campaign.

Chemical Probe:

Compound: a chemical whose structure is known.

Hit: an active well whose positive response is confirmed by replicate runs or generation of a dose response curve using the primary screening assay.

Hit Confirmation: verification of hit activity using an alternative assay to the primary screening assay.

Hit Validation: obtaining compound from vendor or distributor for re-assay, confirming structure of compound by LC-MS and/or NMR etc., and resynthesis of compound if necessary.

Substance: a chemical whose structure is not known or has not been verified by chemical analysis and/or resynthesis; commercially obtained chemicals are substances unless accompanied with the vendors proof of chemical analysis

Assays

Assay Adaptation/Implementation: planning and design phase of process to transfer an assay on to an automated screening system; includes assay and HTS design.

HTS Implementation: the process of transferring an assay on to an automated screening system; includes HTS optimization, modification, miniaturization, intra- and inter-day run variability.

HTS Validation: pre-testing of a new assay using replicate pilot screens of small compound libraries before committing to run the complete screening compound library.

Primary Screening: Initial screen of compound library, usually single dose, to identify active compounds.

Confirmatory Assays: re-screen active wells in the primary assay to verify initial hits. Includes replicate runs and dose response using primary screening assay. This may involve cherry picking hits from primary screen.

Secondary Assays: any assay other than the primary screening assay to gather further information on a hit. Secondary assays can include alternative and counter screening assays. Information obtained from secondary screens may provide hit mechanism of action on target, evaluation of selectivity/specificity of hits, assess agonism vs antagonism, confirm rank order of compound potencies/affinities (e.g. primary: binding assay; secondary: signaling assay).

Alternative Assay: use of a different assay from the primary screening assay to verify the target or mode of action of a hit.

Counter Screen Assays: assays designed to determine the specificity and selectivity of a hit for the target of the primary screen (e.g. other members of receptor or kinase protein family).

Follow Up Assays: the continuation of both the screening assay and counter screening assays at small scale to support chemical optimization of validated hits from the primary screen.

Library Characterization Assay: HTS assay whose purpose is to characterize the physical, chemical or biological property of the compound library (e.g. solubility, toxicity, luciferase activity).

Questions, Comments and Information for applicants

Applicants are encouraged to submit their questions or comments to MLPCN@mail.nih.gov.

Applicants are also encouraged to visit the Molecular Libraries Probe Production Centers X02 FAQ page to find answers to frequently asked questions.

A list of questions specific to each type of Center are below and are followed by general questions about the program announcement.

Answers were provided at the Technical Assistance Teleconference on 6/7/07 at 2 pm EST.

Comprehensive Center

1. What missions or tasks might distinguish the Assay Development Core from the Assay Adaptation/Implementation Core?

   HTS-ready assays for the network are provided through a peer-reviewed submission process (PAR-06-545 (R03) and PAR-06-259 (X01)). The assay development component of the Comprehensive Centers will address improvements to an accepted assay, development work with external scientists to prepare an assay for submission to the MLPCN and assay development by the Center. NIH is looking for indications of experience, and examples of assay development outreach (provide description of previous work with assay providers to aid assay development, and what type of activities and projects NIH would see as meritorious).

2. Will the grant provide for development in the Comprehensive Center? What fraction of resources will go to development of the vision?

   We would like to see fully operational centers apply. The Comprehensive Centers must be at full production by the end of year one and spend less than 10% of the budget in the first year on center development. We expect applicants to provide an operations plan that will provide primary screening of 20-25 assays per year and identification of 10-15 probes per year.

Specialized Center

1. In the RFA instructions, it is stated that "It is expected that the proposed center will have a Principal Director/Principal Investigator for each of the functional cores…" For Specialized Screening Centers, the 4 functional cores are:

   - 1) Assay Development
   - 2) Assay Adaptation/Implementation
   - 3) HTS
   - 4) Informatics.

   Will any alternative organization for a specialized Screening Center be considered?

   Yes. NIH is less interested in the organization of the core pieces, but rather their inclusion of the core functions in the design of their center.

2. Specifically, the PI for the application was responsible for a successful industrial HTS center that was organized in a different manner. All of the same functions were performed. However, the functions of assay development, assay adaptation/implementation and HTS, was performed by a single group or team. The same people performed all of the 3 functions for a single assay. This organizational structure allowed a faster and more efficient completion of each screen. Scientists performing these functions needed additional skills and training initially. Work teams can be organized around each assay, rather than around the functions. Would such an alternate organization be considered?

   This organization is acceptable as long as the functions stated above are inherent in the center organization.

3. Given the fact that at this moment, most of the MLSMR compounds were acquired from commercial libraries, and many of them have other tens or hundreds of available analogs, is a Specialty Screening Center allowed to do cheap and fast post-HTS SAR evaluations of commercially available compounds in order to provide the collaborating Chemistry Centers with better starting points for structure-activity optimization?

   Definitely. This approach is open to all the specialized screening centers, funding permitting. For the specialized screening center, NIH is looking for the center to work closely in collaboration with the specialized chemistry centers. The handoff would probably take place at about the point in probe development where existing compounds of similar structure are tested to provide SAR information.

   When NIH determined the amount of funds for the specialized centers, they did not provide funds for cheminformatics of purchased/synthesis of compounds. Depending on how the center plans to budget the awarded funds, there may be enough for the purchase of compounds. Remember that the highest priority for the center should be to meet their milestones to maintain the funding level.

4. Many of the suggested assay systems and platforms tend to be oriented toward higher content information and are not normally implemented to be high throughput. As assay content increases and newer tools are developed for analysis of networks and pathways are available, general questions emerge.

   - Is the goal to make the currently used high content assays more efficient to garner more information about the chemical-biological interactions, or is it to develop technology to make these assays high throughput?

     Assays for the comprehensive centers must be high throughput. If the throughput is too low, NIH supports any improvement in the process that results in a successful screen. For the specialized screening centers, medium throughput or HTS is acceptable.

   - If the goal is to develop technology, could an emphasis be on technology improvement to enable high content assay development for implementation as higher throughput formats?

     No. The goal is not to develop technology but is to produce probes and we expect a natural evolution of the biology. Assay development outreach is different than what we are talking about here.

5. In light of the issue of content versus throughput, there are questions around the goals of the compound numbers to be screened. For higher content focused screens and emerging or even current

technologies, it will likely be a challenge to get 300,000 compounds in each of 5 assays if these were very high content assays.

- Are the 300k compounds to be tested against each of the 5 assays or a total of 300,000 compounds across all of the assays?

  Screening in the specialized screening center requires 300k compounds for each assay, and as the library grows towards 500k, the emphasis will be on running the full set.

- Are all the library compounds selected to go through the Specialized Screening Centers or could they be a subset of the library already selected by some pre-screens? In other words, will there be a biological hierarchy or logic to organized chemotypes?

  We will emphasize a broad, cross-library data collection. We would like to see what the community considers a specialized capability. Centers can use partial sets of Biofocus/DPI compounds to provide compound sub-sets for developing SAR.

6. In light of a potential focus on higher content assay development, there are issues about preliminary results and ramp-up as part of the process. Will there be sufficient ramp-up time to start with the screening of 10,000 compounds or must the center be HT ready immediately?

   The center must be high-throughput ready and in production mode. We are looking for a center which could do ≥5 assays against 300k compounds in the first year.

7. In light of the multi-center model and the other technology oriented Center and programmatic funding efforts in the NIH Roadmap, many opportunities should exist to share and leverage the expertise through some coordinated vision.

   - Are the screening centers to take the biology of other centers/labs and develop appropriate technology to obtain high content screening from this?

     The centers can do this through assay development outreach. This is a network of centers and collaboration is encouraged through the use of the network Steering Committee. We also encourage assay development and optimization through assay development outreach.

   - What exactly is meant by the review criteria "Are data management and support procedures developed sufficiently to allow tracking of compounds, assays, and screening data"? Is the data management and support to be primarily accounting and tracking, or a more sophisticated system to enable high content analyses and distribution?

     Sufficient informatics is essential to run screens and put the verified/accurate data into PubChem. Centers doing high content screening are responsible for analysis and must set up collaborations with other centers in the network before starting the assay. Centers cannot assume it will be easy to pass an assay to another center. It is important to plan to pass off information to specialized chemistry centers as well.

   - What are the thoughts on utilizing strengths of other centers to enable more efficient screening and data generation in ours? (can we be complementary to other centers?)

     The centers should be self-sufficient, but willing to work with other centers. Centers should be complete in terms of their screening capabilities. They should have the

equipment they need. Credit for primary, secondary and counter-screening can be shared amongst centers involved. We are looking for complementing centers, but the collaboration should be set up ahead of time and the center should have a back-up plan in case the collaborative proposal does not work out.

Specialized Chemistry Center

1. At what point does the Specialized Chemistry Center become involved in the decision making of which hits will be followed up on? Primary screening data, confirmed hits, other?

   The Specialized Chemistry Center begins collaboration with the Screening Center soon after completion of the primary screen and at the time when the screening center begins to decide on compounds to follow-up. When assays are assigned to a specialized screening center, we may want to also assign them to a specialized chemistry center. This maintains the marketplace model approach to get chemists involved right away. The centers need to exchange real data and not PowerPoint presentations. Informatics is needed to upload this data which must have shared file formats.

2. Can the Specialized Chemistry Centers prioritize which hits they will follow up on? Can they reject some confirmed hits as nonstarters? Who has the final say?

   Yes, they can prioritize. This is a joint decision involving biologists and chemists. If there are no hits, NIH may get involved in the decision making.

3. How many screens are the Specialized Chemistry Centers expected to collaborate on per year?

   It depends on the complexity of the screens and type of chemistry needed for follow up; about 10 screens per year.

4. How many probe libraries of ~100-300 compounds are the Specialized Chemistry Centers expected to generate per year? On how many separate confirmed hits?

   About 10 probe projects are expected per year depending on the degree of difficulty in generating the synthetic series of analogs and on the number of synthetic cycles required to find a probe (estimate is that 100-300 compounds will need to be developed and tested per probe project).

5. Will QC and sample amount requirements for synthesized compounds follow the same guidelines as sample submission to the MLSMR?

   Yes. The final probe should be accessible in amounts to allow for further studies by the assay submitter and for submission of at least 15-20 mg to the MLSMR. Appropriate QC methods should be employed to confirm the identity of the compound and to demonstrate the compound is >90% pure. Compounds will be assessed for purity at the MLSMR, using the process in the sample submission guidelines, but may be accepted based on Center-provided purity data.

6. We have a young Center which is growing in the number of staff and equipment we are able to bring on board. What fraction of the application should be devoted to describing current capabilities versus describing the intended strategy/capabilities we would have with a fully funded and staffed Center?

   The center must be fully operational.

7. There are often close analogs available for purchase from external databases. Who would pay for these – the chemistry center or the original screening center?

   The comprehensive center would pay for commercially available analogs if working with a specialized chemistry center for hit optimization. Working with a specialized screening center, the specialized chemistry center would pay for commercially available compounds and for the synthesis of novel analogs.

8. How many screens/projects would the center be expected to supply compound for? Just the primary assay or also for secondary assays?

   The chemistry center will need to provide enough compound to evaluate potency and selectivity of the compound toward the screening target. This would include secondary assays.

9. How much compound would the centers have to make? Should they make enough for all follow-up screening including *in vivo* or just to test the primary screen on a few compounds?

   Centers only need to make enough compound for use within their center, shared with the assay provider and 15 mg must be sent to the MLSMR at Biofocus/DPI.

10. In the "Preliminary Studies/Progress Report" section, what should a new applicant for a specialized chemistry center core describe here? What about for the corresponding administrative/management core?

    NIH is looking for preliminary data and an indication of efficient probe development experience. State your experience with working with biologists and screening scientists. In addition to chemistry expertise, NIH would like an indication that the applicants are able to work with biologists and make the probe development process efficient.

11. Must the experience be at the current center or can the applicant list experience from a previous organization?

    It is up to the investigator as to what experience to list. It would be an advantage to have experience working with the people and equipment at current site, but evidence of any expertise is best.

12. In the "Research Design" and "Methods" sections, the instructions specify a total of 15 pages for Specialized Centers and no more than 5 pages for each core. A specialized chemistry center will be made up of the two cores specified above. This would imply only a maximum of 10 pages for this section. However, since cheminformatics is included within the chemistry core, might it be possible to make the narrative for this core longer than 5 pages? We are thinking of 6-8 pages for the chemistry core. Would this be ok?

    Yes, 15 pages is the total. For the research part of the application, it shouldn't exceed 15 pages, and for comprehensive centers, it should not exceed 25 pages.

13. What is meant by "limited pharmacology" in the expectation of the Specialized Chemistry Centers? Please give examples. Does that mean: in vitro secondary assays that are target-specific?

It is limited to *in vitro* pharmacology within the scope of the definition of a probe and the work necessary to achieve probe status.

It refers to when *in vitro* pharmacology is required or will clearly demonstrate the new probe represents an improvement in the state of the art over existing probes in the literature. If justification to do additional pharmacology is accepted, it can be supported by MLI funds if the pharmacology helps to identify a candidate as a true probe.

General Questions about the Program Announcement

1. About what percentage of the X02s submitted will be invited to submit a U54? How will this look for comprehensive centers, specialized centers, & chemistry centers?

   X02 applicants invited to submit a U54 application will be decided based on scientific merit and their ability to demonstrate that they can meet the production goals set in the RFA. There is not a fixed percentage of invitations to X02 applicants, but NIH expects only a fraction of the X02 applicants to be invited to submit a U54 application. NIH also expects to invite more U54 applicants than will be funded. All three types of centers will be equally evaluated on the same review principles.

2. For the "Specific Aims/Background" and "Significance" sections, the instructions specify 2-5 pages total for these. Is this correct? Are there maximum allowable pages for either of these sections?

   The whole research section is limited to 15 pages for specialized centers (screening and chemistry) and 25 pages for comprehensive centers. The "2-5 pages" is a guideline. Applicants need to clearly identify what they are trying to achieve with their center and be sure to address the goals of the RFA.

3. For the "Resource Sharing Plan," what is the maximum number of allowable pages?

   There is no page limitation.

4. Will the same panel of scientific reviewers review all three types of centers? What will be the professional make-up of the panel?

   Yes, the same panel of scientific reviewers will review all three types of centers. NIH will have a special emphasis panel to obtain the appropriate scientific expertise for the specialized scientific areas expressed in the applications.

5. How will "innovation" be evaluated on these grants?

   Reviewers will receive the RFA and the review criteria as is described in the RFA will be used. Innovation is the criteria in the eye of the reviewer.

6. What is meant by "cost sharing" as described in the Program Announcement?

   "Cost sharing" is what institutional support or resources the proposed center bring to the MLPCN network. Cost sharing is never required but always suggested. It is value added from the NIH perspective.

7. If an organization submits a proposal for a comprehensive screening center at the X02 stage, can/will NIH ask them to re-submit at the U54 stage for a specialized screening center? If so, under what circumstances might NIH make that request?

   The X02 process is an opportunity to get feedback about scientific merit, competency, and the proposed scientific team of investigators. It provides feedback on whether or not the center is strong in all functional areas. If a particular application is weak in several of those areas and looks as though it won't be as competitive as a comprehensive center, but has strong specialized screening or a strong chemistry portion, NIH might provide feedback to add to those strengths.

   It is important to note that a specialized screening center is not a second prize for a comprehensive screening center that doesn't end up being in the competitive range. NIH urges the PI to determine what configuration is their strongest case and lead with that.

8. If a university/institute submits more than one X02 proposal, say both a comprehensive and a specialty screening center, what are the guidelines for proposing non-key personnel in both XO2s?

   "Key personnel" is a term that is defined at the NIH level, but NIH would want the center to propose the research team that is best qualified to fulfill the scientific duty. Applicants should submit a team with each members percentage of participation in the project.

   - Clarification to the question: Would a table that included the percent effort of key personnel be located in the body of the application or as a supplemental document?

     The table can be within the body of the application and not included in the page limit.

9. What are the major differences/functional roles between the Cheminformatics Core within a Comprehensive Center and a Cheminformatics Research Center (PAR-07-353 (X02))?

   The role of a Cheminformatics Research Center is to develop new tools for the analysis of data that is being generated by the centers (beyond commercially available software tools) and also as tools for analyzing the data in PubChem. The cheminformatics cores within the comprehensive centers and specialized chemistry centers will be solely functional. They will be focused on processing and using the tools currently available to process hits, and optimize the hits to probes.

10. In a Comprehensive Center, how many chemists will be needed to do med chem on 25 assays and resulting hits per year?

    It is up to the PI who is writing the application since they know what their capabilities will be. Experience has shown that not all assays will provide reasonable hits for follow up chemistry.

11. Has any decision been made about whether DPI will supply 1 mM or 10 mM stock solutions for the Production Phase?

    At this time, there has not been any final decision made. This is still something the network is trying to optimize during the pilot phase. Any updated information available before the U54 applications are due would be posted on the FAQ page for the U54 RFA.

12. Comprehensive centers should be up to production scale immediately, but for specialized centers who are doing more innovative work, what's anticipated about their ramp up to production scale?

The concept is that specialized centers would focus on assays that are inherently lower throughput but still of moderate screening rate and these centers should also be ready to go from day one with the capability of running 300k compounds on 5 or more assays.

13. Could you comment on how much detail should be given in the application on who will do the follow up assays (secondary, counter-screens)? The X02 at top of p. 7 says "working with the assay provider, valid hits will be characterized in an alternative screen…" Should the MLPCN center be prepared to do all the secondary screens if the assay provider does not? This would require the availability of more post-chemistry biology/pharmacology in the center.

    In the pilot phase, the network relied on the assay PI to carry out alternative screening while the centers were responsible for primary, confirmatory, dose dependence, counter- and follow up screening. NIH recognizes that in most cases the assay PI is the expert in that target or pathway and the PI provides the experience to chose and run the alternative screen. As part of hit confirmation, the results and compounds are shared with the assay PI so they can confirm the results with the alternative screen. Sometimes it is difficult to get the PI to run the assays in a reasonable period of time. In those cases, the alternative screen may be run within the center. This would need to be worked out between the assay PI and the center.

14. Question of current capabilities of the pilot phase verses future capabilities: we could double our chemistry staff in next year; is it fair to write about the productivity we will have at the time of funding instead of what we have currently?

    Yes, that is fine. The important point would be to justify why you think you will have a certain productivity rate and capability by this time next year.

15. Is there any vehicle for subsequent questions prior to X02 deadline?

    Contact Ingrid Li (ili1@mail.nih.gov) or Carson Loomis (loomisc@mail.nih.gov).

16. If you have someone talking about applying for a Comprehensive Center and then realize that their capability is better suited for a Specialized Center and not a production phase Comprehensive Screening Center, would you steer the application that way at the X02 mechanism? Could two X02s join forces and be a comprehensive center on a smaller scale.

    The plans and the metrics NIH is held to are based on completing 20-25 assays per year through a comprehensive center. A comprehensive center will have to conform to that metric.

    NIH encourages centers to apply for one of the three centers and then the X02 review process will allow feedback on the strengths of any given application for the type of center.

    - Clarification of question: Say one group at first wanted to do a Comprehensive Center and then decided to split into a Specialized Screening and a Specialized Chemistry Center, would you encourage the group to apply as a Comprehensive Center or as one or both of the Specialized Centers?

      This is a judgment call on the part of the PI. There cannot be the same PI on multiple applications coming in.

      The goal is to develop a network with the very best resources that are available in the scientific community. If two groups are coming together to have the best resources, then they should apply as one group.

17. What is a ballpark figure for what the funding will be for an individual Specialized Center?

Both the Specialized Screening and Specialized Chemistry Centers will be funded at $3.5M per year (total costs).