| Aim | Task | Year 1 | | Year 2 | | Year 3 | | Year 4 | | Year 5 | | Year 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Aim 1** | **Generation, systematization and dissemination of knowledge about dark kinases** | | | | | | | | | | | | |
| | Coordination with KMC regarding data interfaces and integration | | | | | | | | | | | | |
| | Enhanced outreach/communication of developed resources with community | | | | | | | | | | | | |
| **1.1** | **Prioritizing and systematizing analysis of dark kinases** | | | | | | | | | | | | |
| | Preliminary (existing) prioritization methodology applied | | | | | | | | | | | | |
| | Revised baseline criteria for dark kinase prioritiation defined with KMC | | | | | | | | | | | | |
| | Refined prioritzation methodology established and applied | | | | | | | | | | | | |
| | Broader periodic integration of relevant public data sets (e.g., mutation frequency) | | | | | | | | | | | | |
| | Continued refinement of prioritization methodologies with community | | | | | | | | | | | | |
| | Linkage of prioritization with disease associations | | | | | | | | | | | | |
| **1.2** | **Creating an information resource on dark kinases** | | | | | | | | | | | | |
| | Establish key data and interface variables between DKK and Pharos | | | | | | | | | | | | |
| | Prototype DKK established for testing | | | | | | | | | | | | |
| | Initial functional DKK site public | | | | | | | | | | | | |
| | DKK embeded as a "microsite" within Pharos | | | | | | | | | | | | |
| | Standards development and integration of imaging, dose-response and other data | | | | | | | | | | | | |
| | Iterative integration/linkage of developed tools with KMC websites | | | | | | | | | | | | |
| **1.3** | **Network-level understanding of the dark kinome** | | | | | | | | | | | | |
| | Development and testing of network inference methodologies (proteomic/phospho) | | | | | | | | | | | | |
| | Broader network inference, phenotype linkage | | | | | | | | | | | | |
| | Incorporation of perturbations from later prioritized kinases | | | | | | | | | | | | |
| **1.4** | **Identifying possible therapeutic targets among dark kinases** | | | | | | | | | | | | |
| | Mining of general and specific data sets (e.g., GEO, NHGRI-EBI GWAS, AMP-ADKnowledge) | | | | | | | | | | | | |
| | Community-driven identification of potential disease associations | | | | | | | | | | | | |
| **1.5** | **Reagent validation** | | | | | | | | | | | | |
| | Testing of 101 KO cell lines (HAP1) | | | | | | | | | | | | |
| | Antibody testing (Westerns) | | | | | | | | | | | | |
| | Antibody testing (immunofluorescence) | | | | | | | | | | | | |

**Numbers in year boxes represent number of Dark Kinases (DKs) to be tested**

| | | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | Year 6 |
|---|---|---|---|---|---|---|---|
| **Aim 2** | **Quantitative analysis of DKs using PRM and RNAseq** | | | | | | |
| **2.1** | Selection of DK proteotypic peptides | 150 120 60 | | | | | |
| | DK Survey Assays | 150 120 60 | | | | | |
| | DK Validated Assays | 50 | 50 | 50 | 50 | 50 | |
| | Collaborate with DKK and KMC for analysis of PRM assays | Multiple peptides will be used for each of the 134 DKs so the # of assays for development is > 134 | | | | | |
| **2.2** | **PRM assay of cell lines, primary cells, tumors and tissues** | | | | | | |
| | Initial cell lines will include MCF10A and SUM159 and expanded based on RNAseq | | | | | | |
| | Primary cells incude hES cell, hES-derived cardiomyocytes and neurons, hepato- | | | | | | |
| | cytes and lung epithelial cells and endothelial cells. | | | | | | |
| | Expansion of primary cells such as islet cells, etc. | | | | | | |
| **2.3** | **RNAseq of 36 cell lines and primary cells as in aim 2.2** | | | | | | |
| | Cell lines of different tissue origin | 18 18 | | | | | |
| | Pimary cells of different tissue origin (goal is 10-12 primary cell types) | | | | | | |
| | Collaboration with aim 1, DKK and KMC for DK network analysis | | | | | | |

| | | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | Year 6 |
|---|---|---|---|---|---|---|---|
| **Aim 3** | **Annotate the dark kinome for cellular phenotypes and function** | | | | | | |
| **3.1** | **Development and testing CRISPR reagents for studying DKs** | | | | | | |
| | Prioritize DKs for CRISPR/Cas9 KO based on expression (RNAseq) and mutation | | | | | | |
| | frequency in collaboration with KMC | | | | | | |
| | Target 10 DKs using CRISPR KO in MCF10A and SUM229 cells | 5 5 | | | | | |
| | Target 20 DKs/year in **appropriate cell lines** using CRISPR KO Cell lines to be | | 10 10 10 10 10 10 10 10 10 10 | | | | |
| | defined from ata in aim 2, DKK kinase pages and KMC collaboration | | | | | | |
| | Utilize DKK kinase pages and KMC collaboration to identify DKs for CRISPR-based | | | 3 | 3 | 3 | 3 |
| | mutation and or promoter activation/inhibition (potentially 3 lines per year) | | | | | | |
| | Assay DK KOs for growth/apoptosis/migration/invasion defects | | 10 10 10 10 10 10 10 10 10 10 | | | | |
| | Collaboration with aim 1, DKK and KMC for DK network analysis | | | | | | |
| **3.2** | **Phenotypic analysis of DK perturbation by imaging following genetic or small molecule perturbation** | | | | | | |
| | Genetic perturbations will involve CRISPR/Cas9 KO, mutation or altered expression | | | | | | |
| | developed in aim 3.1 | | | | | | |
| | Small molecule perturbation will be in collaboration with aim 4 as selective | | | | | | |
| | inhibitors are defined for specific DKs | | | | | | |
| | Small molecule perturbation of WT/KO/mutant cell lines | | 5 5 5 5 5 5 5 5 5 | | | | |
| | Small molecule perturbation of primary cells | | | | 2 2 2 2 2 2 | | |
| | Collaboration with aim 1, DKK and KMC for DK network analysis | | | | | | |
| **3.3** | **Reporter-world assay for transcription factor regulation by DK perturbation** | | | | | | |
| | Establish gateway clone set (Flag and BirA*) (30 DKS per 6 months for 2 years) | 30 30 30 30 | | | | | |
| | Gain-of-screen of DKs | | 10 10 10 10 10 10 10 10 10 10 | | | | |
| | Validation studies (QPCR, Western blots) | | | | 10 10 10 10 10 10 | | |
| | Collaboration with aim 1, DKK and KMC for DK network analysis | | | | | | |
| **3.4** | **Determining the effects of DK perturbation on kinome remodeling using PRM** | | | | | | |
| | Homeostasis and adaptive remodeling within the kinome after perturbationby KO | | | | | | |
| | Homeostasis and adaptive remodeling within the kinome after small molecule | | | | | | |
| | perturbation of specific DKs measured by PRM | | | | | | |
| | Collaboration with aim 1 DKK and KMC for DK network analysis | | | | | | |
| **3.5** | **Profiling proteomics and phosphoproteomics analysis will extend PRM-SID assays** | | | | | | |
| | **in aim 2.2. (**Cell lines to be determined from aims 2 and 3 phenotypes) | | | | | | |
| | Collaboration with aim 1, DKK and KMC for DK network analysis | | | | | | |
| **3.6** | **Protein interaction networks** | | | | | | |
| | Establish gateway clone set (Flag and BirA*) as in aim 3.1 | 30 30 30 30 | | | | | |
| | Establish stable cell lines (determined by expression from aim 2, DKK and KMC | | 20 | 30 | 30 | 10 | |
| | collaborations | | | | | | |
| | Protein complex purification and MS | | 10 10 10 | 20 | 20 | 20 | |
| | Reciprocal IP/MS for selected kinases | | 10 | 10 | 10 | 10 | |
| | IP/Western blot validation of top scored candidates | | | 10 | 10 | 10 | |
| | Collaboration with aim 1, DKK and KMC for DK network analysis | | | | | | |
| **3.7** | Metabolic profiling of cells in which DK activity is perturbed | | | 3 | 3 | 3 | 3 |
| | Non-targeted metabolomics to define changes in metabolics with perturbation | | | | | | |
| | of DKs | | | | | | |
| | Collaboration with aim 1, DKK and KMC for DK network analysis | | | | | | |

| | | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | Year 6 |
|---|---|---|---|---|---|---|---|
| **Aim 4** | **Identifying and characterizing cell active chemical tools for dark kinases** | | | | | | |
| | Development of NanoBRET assays for DKs | 30 30 | 30 30 | | | | |
| | Profile literature candidate DK tools | 20 20 | | | | | |
| | Focused screen of priority DKs defined from aims 1-3, DKK and KMC | | 10 10 | 10 | 10 | 10 | |
| | Optimization chemistry of DK small molecule hits | | 10 | 10 | 10 | 10 | 10 |
| | Deliver DK chemical tools | 20 20 | 6 | 6 | 6 | 6 | 6 |
| | **Cumulative** total (DK chemical tools) | 20 | 40 46 | 52 | 58 | 64 | 70 |
| | Collaborate with DKK and KMC for characterization of selective chemical tools | | | | | | |

and phenotypic responses
Make DK chemical tools available to scientific community


**Aim 5** **Collaborations to determine the expression and function of DKs in primary human cells and tissues and with other IDG research groups**
Based on expression profiles defined in aim 2, specific primary human cell populations will be analyzed using DK chemical tools developed in aim 4. Collaborations will be initiated with the IDG awarded research groups with by the NIH oversight committee- projects to be determined.

**Specific Program Milestones**

**Task 1** **Assessment of success rate/throughput of primary technologies** — **End of Year One**
Suggested alterations and revision of milestones by NIH
Approval and acceptance by NIH

**Task 2** **Collaborative plans with RDOC and KMC for DKK** — **Completed by 6-months**
Approval by NIH WG

**Task 3** **With IDG SC minimize and harmonize depositories for data and reagents** — **Complete by end of Year One**
Approval by the NIH

**Task 4** **Assessment of rigor, relaibility, and reproducibility of experiment in each specific aim- from year one and data emerging for year 2** — **Year one data analysis and emerging year two completed by 18 months**
Acceptance of report with required revisions of plan/milestones by the NIH

**Task 5** **Release of initial datasets, reagents, informatic tools, protocols, etc**. — **Completed in Year Two**
Procedures in accordance to IDG SC policies and approved by RDOC and KMC

**Task 6** **Demonstarted success of experimental collaborations via data/reagents other measures in accoradance with IDG SC policies** — **Year 3 milestone**
Approval by RDOC and KMC

**Task 7** **Update of project deliverables, milestones, decision trees, endpoints, timeline** — **End of year milestones**
End of year milestone for years 1-6

Year 1
Year 2
Year 3
Year 4
Year 5
Year 6

Approval by NIH as measure of success for each year 1-6

**Informatics-related**

*"On item #4, this may include other identifiers as determined the IDG SC and NIH (e.g., chemicals registered in PubChem). RRIDs are focused primarily on biological reagents at this time."*

Software applications do have RRIDs (e.g., CellProfiler, ImageJ, etc.) and we will similarly provide software applications with appropriate identifiers.

*"In item #14, we need more information on the informatics development. It is not clear what new analysis tools will be developed or if this an adaptation of LINCS tools. If new tools, what aspects of data analysis will they tackle?"*

**Development of new informatics analysis tools**
While likely relevant to the LINCS efforts, there will be novel computational methods developed specifically for the work proposed here and that will not be developed through LINCS efforts.

- Methods for providing network context to dark kinases. Understanding the potential function/significance of dark kinases will be reliant on understanding their relationship to other kinases, proteins, diseases, phenotypes and relevant functional data. In the context of physical relationships including physical interactions and phosphorylation events, the identification of functional subnetworks is highly valuable. Current methods for identification of related communities (subnetworks) largely rely on approaches that only take into account network topology, but not known signaling pathway relationships and/or other functional behavior. While significant effort within the community is being devoted to understanding the network context of genes and proteins, there is significant opportunity for new and innovative approaches. Similarly, computational methods capable of providing insight into time- and/or condition-specific behavior are greatly needed.
- Methods for linking kinase behavior with the phosphoproteome. Specific to this work, we are investigating novel informatics approaches, for example based on probabilistic graphical models, to infer relationships between kinase data derived from MIB/MS, deep proteomics and phosphoproteomics methodologies. The goal of such informatics approaches will be to establish data-driven functional linkages between dark kinases and substrates, enabling a better understanding of their potential function and their relationship to better-studied signaling pathways.
- System perturbations. Further development of computational methods for the analysis and modeling of network remodeling in response to perturbation - chemical, genetic or other - is needed. In particular, methods for modeling of adaptive kinome reprogramming are needed and will be pursued in this proposal. For instance, the DK and KIN-200 PRM assays provide a unique opportunity to observe reprogramming behavior and computational tools to parse the underlying subnetworks and pathways and their reconfiguration will be required.

- Prioritization. While we have a working dark kinase prioritization methodology in place, numerous algorithms exist for the relative ranking of objects. As knowledge is acquired on dark kinases, we expect that our current prioritization algorithm will need to be modified to incorporate various cost-benefit decisions as well as assist in the potential identification of likely therapeutic targets. As a result, we expect that algorithm development in this area to be one of the new analysis tools developed in the course of this work.