A public-private partnership to unlock the untargeted kinome

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Chemical probes are urgently needed to functionally annotate hitherto-untargeted kinases and stimulate new drug discovery efforts to address unmet medical needs. The size of the human kinome combined with the high cost associated with probe generation severely limits access to new probes. We propose a large-scale public-private partnership as a new approach that offers economies of scale, minimized redundancy and sharing of risk and cost.

Human kinases are pharmacologically tractable proteins that have essential roles in most, if not all, cellular signaling. Despite their central role in biology and their sizeable potential as therapeutic targets, only a small fraction of the 518 human protein kinases have been functionally annotated with 'selective' small-molecule inhibitors[1]. As of September 2012, 15 kinase inhibitors and 5 antibodies have been approved as drugs in the USA, involving merely nine different kinases as primary targets, according to the US National Cancer Institute (http://cancer.gov/cancertopics/druginfo/alphalist/). Some scientists believe that most of the therapeutically relevant kinases have been studied, but numerous lines of biological evidence strongly suggest that the unexplored kinome contains new opportunities to address unmet medical needs in cancer, metabolism, inflammation and other diseases[1].

Reflecting a dynamic observed in research on the broader human genome[2], a key reason for the continued failure to fully appreciate this enormous therapeutic opportunity is the lack of quality chemical probes that would enable the biological evaluation and pharmacological understanding of the untargeted kinome. Biological tools and techniques to study the entire kinome are available and essential in defining kinase function. However, in many contexts, the application of small-molecule approaches offers considerable advantages in experimental demands and interpretation of results. For example, RNAi techniques and genetic knockout or knock-in models are limited by the kinetics of their effects and the inability to discriminate between scaffolding and catalytic roles of the target protein. Although some of these limitations can be overcome (for example, RNAi plus rescue experiments with catalytically dead and wild-type kinases), such efforts can be technically complex and challenging to execute.

The kinome is not unique in needing quality chemical probes. Other established protein families for drug discovery, such as ion channels, G protein–coupled receptors and nuclear receptors, are in similar need of a comprehensive set of small-molecule tools. The kinome in particular, however, has features that augur well for the efficient development of chemical probes. First, there is a rich pharmacopeia of kinase inhibitor templates within industry. Second, most small-molecule kinase inhibitors target the ATP-binding site, and, because the structural features of ATP binding are conserved, they can serve as starting points for selective inhibitor design. Third, there is structural information for a large number of protein kinases, providing the opportunity to use structure-based design. Fourth, there are large numbers of kinome-wide assays that can be used to aid in designing potent and selective inhibitors, such as kinase assay panels and techniques to probe for inhibitor activity and selectivity in cells. The biochemical assays vary in a number of different experimental parameters, including constituent kinases, expression constructs, assay technology, assay conditions, kinase and ATP concentration and kinase substrate selection (when applicable). Cell-based techniques include multiplexed chemical proteomic competition binding assays, which use resins appended with promiscuous kinase inhibitors to affinity-capture target kinases from the cell lysate[3], and activity-based profiling, which presents a complementary method in which, for example, highly reactive biotinylated acyl phosphate derivatives of ATP are used in affinity-tagging the catalytic lysine of native target kinases from cells[4].

The large number of reported inhibitors and heterogeneity of associated data create a need to standardize criteria for selecting and properly using kinase chemical probes. It will be critical to use stringent kinome-wide biochemical and cell-based assays to characterize chemical probes, including those partially characterized probes already described in the scientific literature and available from commercial sources. Moreover, there is value in profiling potential probe molecules beyond the protein kinome as cross-reactivity has been documented with the related phosphoinositide kinases as well the more distant G protein–coupled receptor superfamily[5,6]. Many claims about the utility of reported compounds do not hold up to scrutiny because they are based on incorrect or insufficient characterization of inhibitor selectivity and cellular potency. Indeed, recent surveys of reported kinase compounds revealed that claims of selectivity are frequently overstated, which substantially limits the utility of many of these probes[5,7].

Frye has proposed that a quality chemical probe must have a known selectivity profile, a well-established mechanism of action and a known active species; have demonstrated cellular activity to confidently address hypotheses about the activity of its target;

Table 1 | Examples of existing high-quality kinase probes.

Compound	Target kinase	Inhibition mechanism
Protein kinases		
CGI1746 (ref. 14)	BTK	ATP competitive
SGX523 (ref. 5)	MET	ATP competitive
LRRK2-IN-1 (ref. 15)	LRRK2	ATP competitive
GSK2334470 (ref. 16)	PDK1	ATP competitive
Selumetinib[a] (ref. 5)	MEK	Allosteric
GW2580 (ref. 5)	cFMS	ATP competitive
JNK-IN-8 (ref. 17)	JNK	Covalent
GNF-5 (ref. 18)	ABL	Allosteric
KH-CB19 (ref. 19)	CLK1/4	ATP competitive
ML167[b]	CLK4	ATP competitive
NIH CLK/DYRK[20]	CLK/DYRK	ATP competitive
Ruxolitinib[5]	JAK2	ATP competitive
Tofacitinib[5]	JAK3	ATP competitive
Lapatinib[5]	EGFR/ERBB2	ATP competitive
Afatinib[5]	EGFR/ERBB2	Covalent
BI2536 (ref. 5)	PLK1	ATP competitive
GSK461364 (ref. 5)	PLK1	ATP competitive
MK5108 (ref. 21)	Aurora A	ATP competitive
VX-745 (ref. 5)	p38α/β	ATP competitive
Skepinone-L[22]	p38α/β	ATP competitive
MLN-120B[5]	IKKβ	ATP competitive
GDC-0879 (ref. 5)	BRAF	ATP competitive
Phosphoinositide kinases		
CZC24832 (ref. 23)	PI3Kγ	ATP competitive
GDC-0941 (ref. 5)	Pan-PI3K	ATP competitive
KU-60019 (ref. 24)	ATM	ATP competitive

[a]A number of highly selective inhibitors have been identified that target an allosteric binding site in MEK. [b]See http://www.ncbi.nlm.nih.gov/books/NBK47352/.

and be readily available[8]. Guided by these principles, we undertook an assessment to determine how many reported kinase inhibitors could actually be confirmed as quality kinase probes. The criteria used were (i) potent inhibition of primary target (or targets) with at least 50-fold selectivity over other targets, (ii) the availability of selectivity measurements on at least 25% of the human protein kinome or a selectivity profile measured using a chemoproteomic method and (iii) demonstrated cellular activity data to confidently support usage as a probe. We emphasized kinome selectivity profiling in particular because the majority of these inhibitors target the ATP-binding site, and selectivity issues within the 'chemically connected' kinome have been well documented. Our survey revealed very few probes that are sufficiently well characterized to meet these criteria (**Table 1**) and puts in question the conclusions of a large swath of kinase literature.

The emphasis on high-quality chemical probes is not intended to discount the utility of compounds that fail to meet these stringent criteria. In practice, compounds that fall short of being bona fide kinase probes frequently have value as pathfinder compounds in a new area or as iterations toward a high-quality inhibitor[9]. For each target under consideration, a value chain can be envisioned for compounds and is informed by available characterization data (**Fig. 1**).

Chemical probes will be essential to accelerate understanding of the human kinome, and the existing chemical probe arsenal is both small and inadequate for the task. We believe that the tools and expertise to efficiently and rationally generate chemical probes are now available. In addition to the size and complexity of the kinome, a key challenge is that no single company or any individual academic group can take on this costly task alone. Neither industrial nor academic funding schemes have established mechanisms to generate research tools for proteins that are understudied. These organizational hurdles present barriers to our ability to understand normal physiology and disease pathology and to identify new targets and mechanisms for therapeutic intervention. Traditional approaches in preclinical drug target validation have created and preserved an untargeted kinome, so a new approach is required to realize the therapeutic value of targeting new kinases. We believe that the only path forward is to pool resources and share risks. Although several models for increased collaboration have been considered[10], we propose the formation of an open public-private partnership (PPP; **Box 1**) whose aim is to facilitate the understanding of the human protein kinome through the generation of high-quality, freely available chemical probes and the provision of sufficient information to enable the proper usage thereof.

A kinase chemical probe partnership

An ideal kinase chemical probe partnership would rapidly generate high-quality chemical probes in the most cost-effective way and would ensure open access to these tools and all standardized supporting data. In addition, the partnership would support the annotation of the probes following their release into the scientific community through a searchable database that catalogs biological activity across cellular and biochemical assays. An effective means of achieving this goal would use capabilities currently available in both industry and academia. These resources would be supported with additional platforms that are typically inaccessible to or overly expensive for individual investigators. For instance, the large number of academic chemists working on kinase inhibitor design cannot afford costly commercial screening platforms for biochemical and cellular testing. We envision, therefore, that a crowd of medicinal chemistry efforts both from within and external to the PPP would be supported by platforms from the partnership, each serving as a centralized locus of expertise and resource in an area essential for the overall effort. These platforms would include protein screening, structure determination, cellular testing, data management and probe dissemination. The organization of the effort into platforms would allow for efficiency of scale, minimize duplication of effort and create opportunity for specialization. Additionally, past experience from large-scale ventures (for example, the Human Genome Project) suggests that highly specialized centers become hubs of technological innovation because the development of new technologies is required to meet their ambitious goals.

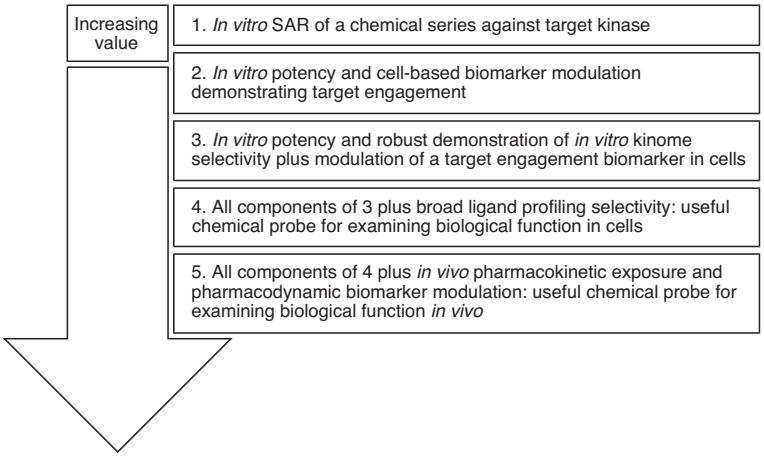

Figure 1 | Value chain of kinase inhibitors based on validation for biological investigations. *In vitro* profiling refers to biochemical or cellular assays, and *in vivo* screening describes studies conducted in multicellular organisms. SAR, structure-activity relationship.

Multifaceted interinstitutional and international collaborations can be crippled by legal negotiations among the parties. To minimize this complexity, we suggest that the chemical tool compounds should be generated and distributed without intellectual property burdens whenever possible. Consistent with this position, the partnership could agree to screen any chemical matter if the provider agrees to release the probe compound without restriction on use for research purposes. Several academic investigators, including the coauthors of this paper, endorse this position as an expeditious means to provide these tools to the research community. This 'open-access' position will facilitate the formation of a large network of collaborators and would align with other efforts whose objectives are to generate chemical probes, such as the Structural Genomics Consortium (http://www.thesgc.org/) and the Molecular Libraries Probe Production Centers (http://mli.nih.gov/mli/). In both of these projects, geographically distributed groups function in independent but aligned fashions to meet specific goals and milestones.

Starting chemical matter

The development of chemical probes is critically dependent on the quality of the chemical matter that feeds the platforms. With kinases, chemical starting points often emerge from libraries designed to target the conserved ATP-binding site. Several groups have verified experimentally that inhibitors of previously untargeted kinases can be identified through screening of small sets of ATP-competitive inhibitors. Libraries comprising these types of inhibitors are widely available in industry, and the PPP will access them from participating companies. Indeed, this process has already begun; GlaxoSmithKline and Roche have made freely available sets of hundreds of kinase inhibitors from previously published accounts of proprietary drug discovery efforts, on the condition that any data generated from its use are made publicly available. Other pharmaceutical companies have expressed the desire to release additional kinase inhibitor sets to external investigators. The PPP may also expand its chemical libraries to include commercially available inhibitors, natural product sets, fragment sets and other compound collections supplied by industry or academia. Alternatively, the PPP may draw on institutional knowledge in template design to prepare screening sets containing new inhibitor motifs.

The platforms will also be positioned to identify probes with diverse inhibition mechanisms. Although inhibitors that bind in the kinase ATP-binding site might be the initial focus of the effort, allosteric inhibitors may offer more favorable opportunities to achieve selective inhibition and should be an important additional objective. Many assays can identify inhibitors irrespective of mechanism, and there are assay strategies that look specifically for allosteric inhibitors. Co-crystal structure determination might also facilitate the generation of allosteric probes and contribute to the understanding of their inhibition mechanism.

Probe generation

The transformation of a chemical hit or starting point into a high-quality chemical probe relies on iterative medicinal chemistry. Previous efforts to optimize kinase ligands have defined strategies for the attainment of highly selective inhibitors. Additionally, ligand-bound crystal structures constitute an informative and rational basis for the design of inhibitors with increased potency and selectivity. The dedicated medicinal chemistry platform of the PPP itself will be complemented by contributions from industrial and academic partners. Importantly, the open approach proposed herein, which would allow plans to be crowdsourced, is expected to increase the quality and efficiency of probe generation.

Probe criteria

We propose to adopt the principles of Frye[8], which pragmatically eschew rigorous and proscriptive quantitative probe criteria to avoid exclusion of potentially useful compounds. The enumeration of quantitative guidelines, however, is useful. In addition to the considerations from Cohen[11] as well as the chemical probe fitness factors of Workman and Collins[9], we suggest that the

Box 1 | Why an open PPP?

Taking on the kinome within an open PPP offers several advantages to traditional approaches, many of which derive from the scale of achievable activity that would not be possible within any single organization. The PPP format provides the opportunity to mix the diverse expertise and capabilities of industry and academia in the pursuit of a common goal, where the wealth of knowledge and expertise in academic biomedicine can be complemented with the experience of industry in medicinal chemistry and the delivery of milestone-driven goals. By forming hubs of expertise within the PPP, resources and capabilities can be used more efficiently. Moreover, industry brings to the PPP resources, such as strategic compound sets, which are difficult to obtain in the academic sector. By operating in the open with transparent goals and minimizing intellectual property burdens, access to and participation of the world's top scientists is facilitated. The ability to share both risk and cost makes the total project less expensive than having constituents independently generate tools. At the same time, the open operations of the PPP allow funds to be used more efficiently, owing to decreased redundancy.

A potential criticism of the open PPP model is that it will prevent commercialization; on the contrary, the PPP model fertilizes proprietary kinase drug discovery efforts through increased quality of preclinical kinase target validation, which is a known bottleneck in the drug discovery process.

Box 2 | Why selective kinase inhibitor probes?

Single-target kinase inhibitors have been criticized as overly simplistic because many of the observed clinical effects of kinase inhibitors (for example, imatinib and sorafenib) can be accurately ascribed to polypharmacology. It is important to keep in mind that the multitargeted kinase inhibition profiles of these compounds most often originated from serendipity rather than rational design. Moreover, arguments for polypharmacology fail to recognize the difference between a probe compound developed as a useful tool to interrogate biology and one developed as a drug designed to induce a clinical effect. An understanding of the underlying biology is needed to arrive rationally at a multitargeted inhibition profile. It is this fundamental understanding that is enabled by selective chemical probes. The selective probes themselves may be clinically effective and lead to innovative new medicines. In addition, high-quality, selective chemical probes can also define desirable polypharmacological profiles or suggest combinations of selective agents to use clinically[13].

ideal kinase chemical probe should have a cellular potency well below 500 nM and have selectivity at least 100-fold over other kinase and nonkinase targets (**Box 2**). Unfortunately, this degree of potency in cells and selectivity against other targets is difficult to obtain, owing to a high level of conservation of the kinase catalytic domain and the presence of often closely related isozymes. If a compound were to meet these guidelines, it would clearly be a useful probe. Compounds that are close to these criteria may also be very valuable as tools to elucidate biology so long as their activity profiles are well defined[9]. Thus, the comprehensive characterization of a probe molecule in biochemical and cellular studies remains the most critical quality criterion for the partnership.

Independent probe assessment

The medicinal chemistry platform and external chemists will optimize compounds for activity, selectivity and other properties. Every candidate molecule being considered as a probe should proceed through a defined set of cellular assays, such as cell proliferation in panels of molecularly characterized cancer lines from the US National Cancer Institute, the Sanger Institute or the Institute for Cancer Research, London. The cellular responses of probes can be observed and interpreted in the context of known kinome inhibition profiles and other characterization data. To avoid the potential for conflict between compromising probe quality and the PPP meeting its goals for numbers of probe molecules generated, an independent board of scientists will assess the quality of the probe before its release to the community.

There are compelling reasons to fully characterize newly generated probes. These same reasons are applicable to the evaluation of previously reported kinase inhibitors. In many cases, inhibitors with activity profiles have been described with encouraging yet nonetheless insufficient functional characterization to determine the inhibitor's utility as a probe. A recent example of such a compound is a selective CK2 inhibitor; the study reports profiling of the key compound across a panel of 324 kinases but describes concentration-response data for only 4 of the 16 compounds that showed more than 50% inhibition at 1 μM[12]. The PPP will proactively identify compounds for further study but also consider requests from investigators wanting further characterization of their best compounds. In some cases, the additional data generated by the PPP may further enable the usage of the compound, and other times it may identify issues to address with iterative medicinal chemistry.

Probe distribution

Chemical probes can make an impact only if they are readily available in amounts sufficient for biochemical and cellular studies at minimal cost and with no restrictions on usage. To accomplish this, the initiative will build on pre-existing compound distribution networks and capabilities provided by several commercial suppliers. Material provision of a chemical probe is insufficient to ensure that it is optimally used by the research community. It is also necessary to ensure that the community has open access to probe characterization data. The PPP must make available the probes themselves as well as the accompanying data packages. Each of these web-accessible data packages will minimally include a standardized battery of characterization, and each probe will have additional data specific to the targeted kinase. For example, the probe might optimally be used with a structurally related inactive compound and/or a second probe of a distinct chemotype that is active against the same molecular target[9].

Data

The standard data package supplied with a kinase probe will constitute only a small fraction of the data generated and made available by the PPP. In addition to extensive characterization of the final probe, full details of the scientific path from which the probe was delivered will be accessible, including all intervening compounds, associated assay data and protocols. The ability to search the data by kinase, assay type and chemical structure and to visualize and interact with data in a straightforward manner is essential. Recognizing that simple provision of data is insufficient to enable its optimal use, the Kinase SARfari chemogenomics workbench (https://www.ebi.ac.uk/chembl/sarfari/kinasesarfari/) on the European Bioinformatics Institute's ChEMBL database has been developed as an interactive data repository specifically for kinase-related data and could be a useful online host for the data generated by the PPP.

Given the size of the kinome and the need to generate hundreds of inhibitor probes, the scope of the kinase chemical probe partnership will be unprecedented. However, its size is neither the only nor the most important distinction from previous efforts to generate chemical probes, such as the Structural Genomics Consortium–led epigenetics initiative[10]. In addition to building on a substantial knowledge base from over a decade of large industrial investment in kinase research, the kinase chemical probe partnership will more fully embody open innovation. For example, medicinal chemistry plans and data interpretation will be crowdsourced, allowing input from a diversity of perspectives and access to otherwise difficult-to-obtain institutional knowledge. Moreover, in an empirically driven pursuit such as chemical probe generation, much value can be derived from unpublished and negative results, which are typically not captured in an accessible fashion. The totality of the data combined with the diversity of views and the size of the contributing pool of scientists may enable solutions to historically difficult problems, such as the generation of allosteric kinase inhibitors.

The effort described herein will generate a publicly available well-characterized 'tool kit' of chemical probes that will cover the unexplored targets of the kinome, one of the most successful target areas in drug discovery. The results will be compiled to grow a database associating chemical inhibition of kinases to phenotypes and to contribute to the systems understanding of signaling networks. Ideally, kinases or combinations of kinases will be linked with therapeutically

relevant outcomes, both desired and undesired, in a reasonable amount of time[13]. We believe this broad and unbiased approach will identify therapeutic opportunities across the kinome and revitalize kinase drug discovery efforts. In addition, some of the chemical probes themselves may serve as starting points for drug discovery. Although the probes are the direct output of the partnership, the ultimate measure of success will be addressing unmet medical needs. ■

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Competing financial interests

The authors declare competing financial interests: details are available at http://www.nature.com/doifinder/10.1038/nchembio.1113.