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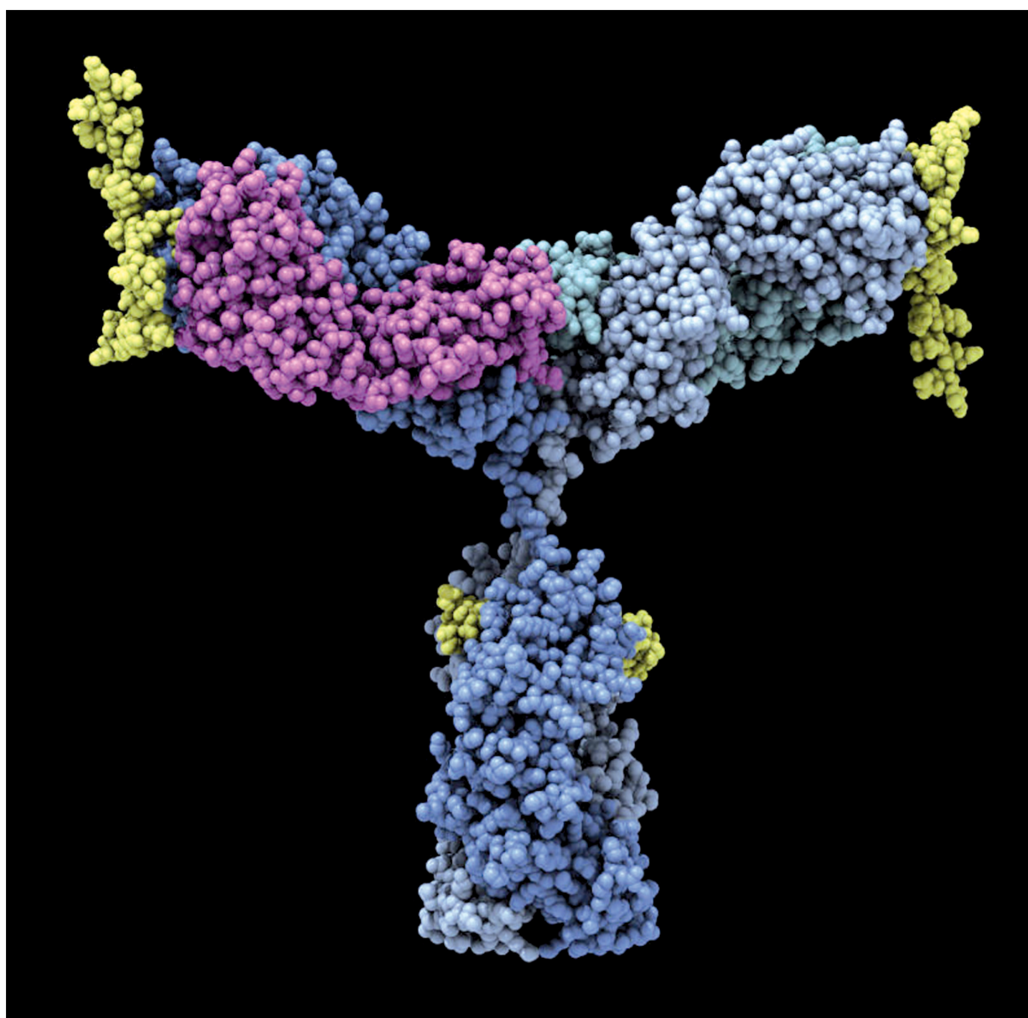
New Frontiers in Chemical Biology

Enabling Drug Discovery



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Preface

"Few scientists acquainted with the chemistry of biological systems at the molecular level can avoid being inspired."

Donald J. Cram

Despite ever-increasing investment in biomedical research, there has been a significant decline in the number of new drug approvals in recent years. In an effort to improve productivity, and expand accessible drug space, the drug discovery community has increasingly looked to advance alternative therapeutic paradigms, such as biotherapeutics (antibodies, vaccines, nucleic acids, peptides, *etc.*), in addition to more classical small molecule medicinal chemistry approaches. However, independent of chosen therapeutic modality, improving future drug discovery productivity also critically depends on reducing the high levels of attrition currently seen in "proof-of-concept" Phase II clinical trials. This will require the development of a much deeper knowledge of biological systems in order to identify and validate those biomolecular targets for which there is the highest possible confidence of disease-relevance in humans.

Chemical biology is an emerging field at the interface between chemistry and biology that utilises the tools and techniques of chemical synthesis to study and influence biological systems. Recent developments in chemical biology have great potential to help address the drug discovery productivity challenges expressed above. For example, chemical biology studies have already led to the identification of novel targets with exciting therapeutic potential and it is clear that the field will prove a key enabler of improved target discovery and validation in the future. Moreover, the precise synthetic manipulation of biological molecules germane to many chemical biology approaches is also now fuelling a new wave of chemically modified biologics with unique properties ("chemologics") that emerge from the union of medicinal chemistry design with biotherapeutics research. In these, and many other ways, chemical biology is

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beginning to emerge as a key discipline within 21st century drug discovery and the purpose of this book is to highlight a number of the important developments in this regard. Since the field of chemical biology is both vast and growing, it should be stressed that this book is not intended to be exhaustive. Instead, the combination of general overviews in this book, coupled with the more in depth treatments of selected topics, is intended as an introduction to the field for the reader.

I would like to thank all of the contributors to the chapters in this book for their outstanding efforts and support of this project. I would also like to thank the staff at RSC, especially Gwen Jones and Katrina Harding, for their patience and support in bringing the book to completion. It is very much hoped that this book will provide a useful resource for scientists, both in industry and academia, who are looking to build their awareness of chemical biology and how this exciting field of science may enable future drug discovery.

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CHAPTER 1

The Chemical Genetic Approach: The Interrogation of Biological Mechanisms with Small Molecule Probes

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1.1 Introduction to Chemical Genetics

The chemical genetic approach[1] exploits small molecule probes to modulate the function of biological macromolecules, and hence to reveal insights into biological mechanisms. The term ''chemical genetics'' stems from the analogy of the approach to classical genetics. Chemical genetics exploits small molecule probes to modulate protein function; in contrast, classical genetics modulates protein function indirectly through mutation of the corresponding gene. The features of the chemical genetics and classical genetics are rather different, making the two approaches broadly complementary.

The use of small molecule probes can confer a number of advantages over conventional genetic manipulations. First, the effects of small molecules on the function of a target protein are conditional and, in addition, are usually rapid (usually diffusion-controlled) and reversible.[2] Second, by varying the

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concentration of a small molecule probe, it may be possible to tune the activity of a target protein in a refined way.[3] Third, the chemical genetic approach can sometimes allow multiple functions of an individual protein to be independently modulated.[4,5] Fourth, by using more than one small molecular probe in combination, the function of multiple proteins may be independently modulated.[6] Finally, the approach is useful when classical genetics is difficult to employ: for example, in organisms (especially mammals) with diploid genomes and a slow reproductive rate.[7] The chemical genetic approach is particularly useful for investigating tightly coordinated, dynamic biological mechanisms, especially when classical genetics may render an organism inviable.[8]

One of the obvious features of classical genetics is its incredible specificity: mutation of a specific gene can allow a single protein (from a family of closely related proteins) to be targeted. In addition, classical genetics is highly general, and can be used to study the function of many proteins. However, classical genetics does not generally allow the *conditional* modulation of a specific protein (unless, for example, a temperature-sensitive[9] mutant is used). RNA interference (RNAi) is a popular alternative to classical genetics which works by inhibiting the synthesis of a specific protein by targeting a specific RNA transcript.[10] However, RNAi is also poorly suited to time-sensitive studies because the modulation is made at the mRNA level: degradation of the protein target needs to occur before the effects of modulation are observed.

The chapter is not an exhaustive review of chemical genetics: the field, and its impact on our understanding of fundamental biological mechanisms, has already been comprehensively reviewed elsewhere.[1] Rather, the chapter will emphasise the approaches that may be used to discover small molecule probes, the solutions that address the challenges raised by chemical genetics, and the insights into fundamental biological mechanisms that may be revealed.

1.1.1 Forward and Reverse Chemical Genetics

The chemical genetic approach comes in two broad guises that are contrasted in Figure 1.1: forward chemical genetics and reverse chemical genetics.[1] In the forward chemical genetic approach, the target of the small molecule modulator is not known at the outset of the investigation. The active ligand is, therefore, identified on the basis of the observation of a specific phenotypic change that it induces. A major challenge of the forward chemical genetic approach is the subsequent identification of the protein that the active ligand targets.[11] The interrogation of biological mechanisms using the forward chemical genetic approach (and the associated challenges associated with target identification[11]) are described in Section 1.3.2. The forward chemical genetic and the forward genetic approaches are broadly analogous in that a phenotypic change to a biological system is induced in both cases: through modulation using either small molecule probes (sometimes known as "perturbogens"[12]) (forward chemical genetics) or random mutagenesis (forward genetics).

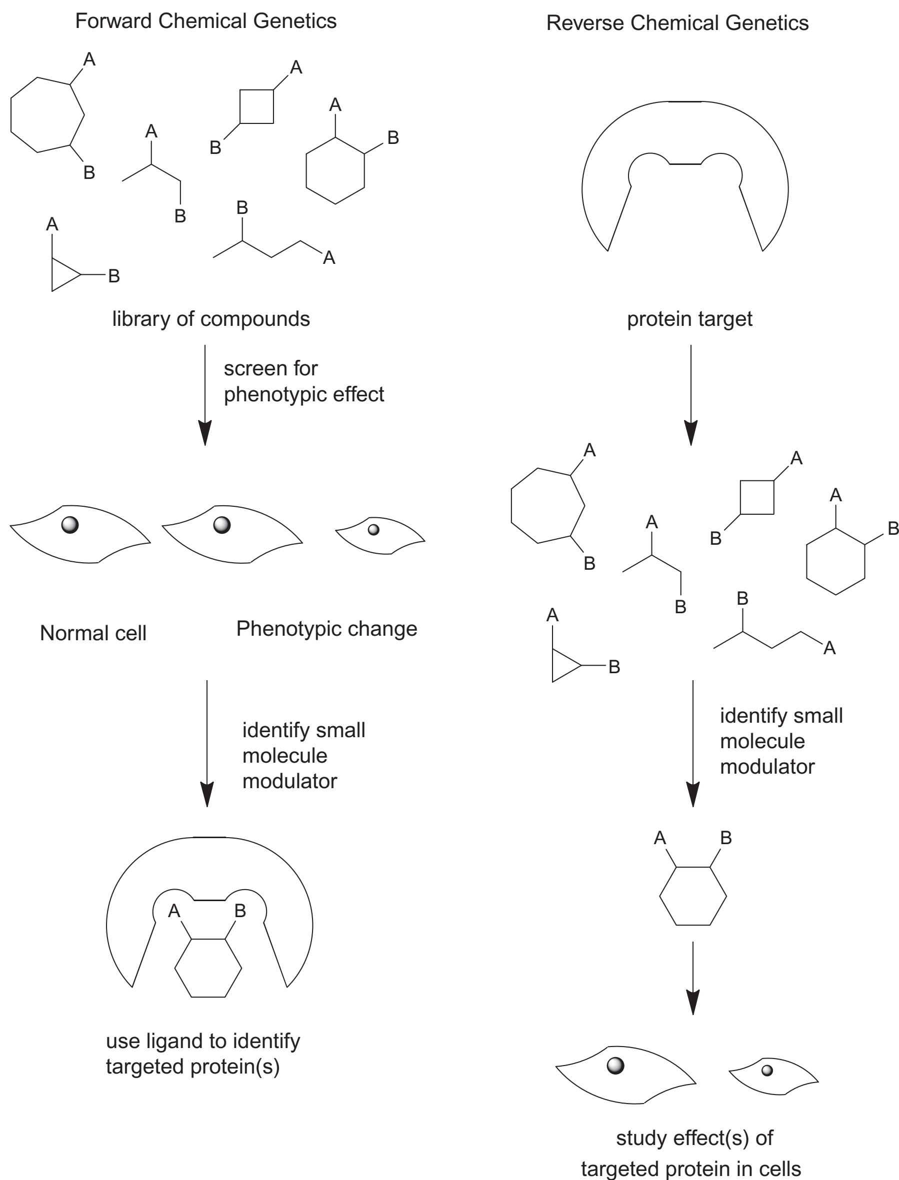


Figure 1.1 Schematic overview of forward and reverse chemical genetics. In forward chemical genetics (left panel), a small molecule probe is discovered on the basis of its phenotypic effect; the small molecule probe may then be subsequently used to identify the protein responsible for the phenotypic effect. In contrast, in reverse chemical genetics (right panel), a small molecule probe is discovered on the basis of its ability to modulate a specific protein; the small molecule probe may then be used to modulate the cellular function of that specific protein, and the phenotypic effects that result.

In contrast, the reverse chemical genetic approach begins with the discovery of a small molecule modulator of a specific target protein. Most usually, the small molecule is identified on the basis of the modulation of the activity of the purified protein; however, assays of the cellular activity of a specific protein are also possible. Both reverse chemical genetics and reverse genetics modulate the function of a specific protein: either directly by the binding of a ligand (reverse chemical genetics), or indirectly through targeted mutagenesis of the corresponding gene. The discovery of ligands for use in reverse chemical genetics, and some of the insights into biological mechanism that have thereby been revealed, are described in Section 1.3.1.

1.1.2 Screening Small Molecule Libraries: Some General Considerations

The forward and reverse chemical genetic approaches both require the identification of an appropriate small molecule modulator. Most usually, active ligands are initially discovered using a high-throughput primary screen. In such cases, it is, of course, imperative that active molecules can be reliably distinguished from inactive molecules, and the performance of an assay must be assessed using appropriate statistical parameters (such as plate-based *Z* factors).[13] In addition, it is necessary to correct screening results for systematic error by normalisation to controls (for example, to controls on each screening plate).[14] Although guidelines for reporting data from high-throughput screening of small molecule libraries have been suggested, appropriate standards have not yet been formalised.[15]

Having identified a "positive" in a primary screen, further investigation is necessary to confirm its validity.[15,16] Greater confidence is gained if an active compound is related structurally to other active compounds identified in the assay (allowing preliminary structure-activity relationships to be formulated). It is important to confirm that the active compound did not interfere with the primary screen: possible problems can include inhibition of a coupling enzyme in an assay (rather than the target protein), or the interference of a fluorescent compound with a fluorescence-based readout. The chemical structure of the active compound must be verified, and then, ideally, resynthesised, repurified and retested. Ideally, the activity of the compound should be confirmed using one or more independent assays.

1.2 Synthetic Strategies for Exploring Biologically Relevant Chemical Space

A challenge in chemical biology is to design small molecule libraries that span large tracts of biologically relevant chemical space.[17] The chemical space defined by small molecules is vast: it has been estimated that there are $>10^{60}$

compounds with molecular weight less than 500 Da (only a tiny fraction of which have been prepared by chemical synthesis).[18] How then, can the biologically relevant sub-fractions of chemical space be identified and targeted?

One approach is to design libraries around known scaffolds that have been biologically validated such as those found in drug molecules[19] or natural products.[20] In biology-oriented synthesis (BIOS),[20] for example, natural product scaffolds are regarded as pre-validated starting points for ligand design since they have been selected through evolution to interact with protein binding sites. In a related approach, libraries may be designed around scaffolds that are closely related to those that are already known to be biologically relevant.[21]

Alternatively, a fragment-based approach may be used in ligand design.[22] Essentially, a fragment-based approach dramatically reduces the chemical space that needs to be searched. Emphasis is placed on ligands with high "ligand efficiency" (binding energy per heavy atom) rather than high absolute affinity. The approach allows a much higher proportion of the relevant chemical space to be explored, whilst leaving ample scope (and providing a better starting point) for ligand optimisation.

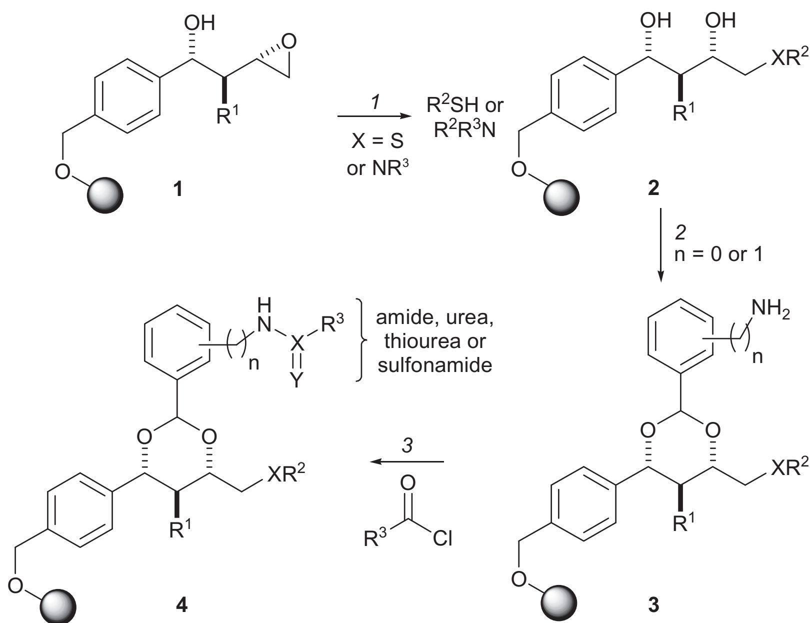
The diversity of chemical libraries may derive from the high substitutional, stereochemical and scaffold diversity of its members. Combinatorial chemistry is focused on the synthesis of chemical libraries with only high substitutional diversity. In general, combinatorial syntheses exploit a rather limited palette of chemical transformations to vary the substitution of library members. The synthesis of compound libraries with high substitutional diversity is described in Section 1.2.1.

Varying the configuration of library members is more challenging, but has been hugely facilitated by the many reliable asymmetric transformations that have been developed in the past 30 years. In many cases, the high enantioselectivity of these transformations can dominate over substrate-based diastereoselectivity, and allow the configuration of compounds to be varied systematically. The synthesis of a compound library with both high substitutional and stereochemical diversity is described in Section 1.2.2.

Charting chemical space systematically, however, does require the preparation of libraries with high scaffold diversity. Historically, chemists' exploration of chemical space has been highly uneven and unsystematic, and much emphasis has been placed on a small number of molecular scaffolds.[23] (Note that the ~30 million cyclic organic compounds in the CAS registry are overwhelmingly dominated by a remarkably small number of molecular scaffolds:[1] thirty (of the 2.5 million!) molecular scaffolds are found in 17% of the compounds, and 0.25% of the scaffolds are found in 50% of the compounds.) A key goal of diversity-oriented synthesis[24] (DOS) is to address this problem through the preparation of libraries of small molecules that populate broad tracts of chemical space. In Section 1.2.3, the progress that has been made towards developing synthetic strategies that allow the systematic variation of scaffolds of ligands will be described.

1.2.1 Synthesis of Compound Libraries with High Substitutional Diversity

The combinatorial chemistry approach has been extended to the diversification of complex scaffolds, including natural product[25] and natural product-like[26] scaffolds. For example, a library of conformationally restricted 1,3-dioxanes **4** with high substitutional diversity has been prepared (Scheme 1.1).[26] A split-pool approach was used to diversify three substituents in the final compounds **4**. The supported epoxides **1** were opened with either a thiol or an amine nucleophile to yield 1,3-diols **2**, which were converted into Fmoc-substituted 1,3-dioxanes. Some of the nucleophiles used in the first step were hydroxy-substituted, leading to the formation of mixed acetals in the second step: these acyclic acetals were, therefore, hydrolysed by treatment of the resins with PPTS in THF-MeOH. Following removal of the Fmoc group (→ **3**), the resulting free amines were converted into amides, ureas, thioureas or sulfonamides (→ **4**), and the final compounds cleaved from the beads. Using material cleaved from a single bead, and with knowledge of the substituent added into the final step, it was possible to use mass spectrometry to identify possible combinations of substituents added in the first two diversification steps. The library has been exploited in the discovery of small molecule tools: uretupamine



Scheme 1.1 Schreiber's split–pool synthesis of 1,3-dioxanes with high substitutional diversity. *Reagents and conditions*: (1) R^2SH or R^2R^3N; (2) (i) $ArCH(OMe)_2$, Me_3SiCl, HCl, dioxane; (ii) PPTS, THF–MeOH; (iii) piperidine; (iv) Me_3SiCl; (3) amide, urea, thiourea or sulfonamide formation.

(see Section 1.3.1.1),[27a,b] which targets Ure2p, a repressor of metabolic genes, and tubacin,[27c,d] a class II histone deacetylase inhibitor. In addition, derivatisation of the scaffold of the alkaloid, galanthamine, yielded secramine, a potent inhibitor of protein trafficking from the Golgi apparatus to the plasma membrane;[25] crucially, although secramine's structure was inspired by galanthamine, it had an entirely distinct biological function.

1.2.2 Synthesis of Compound Libraries with High Stereochemical Diversity

The synthesis of stereochemically diverse compound libraries has been facilitated by the development of a wide range of reliable asymmetric transformations. For example, an asymmetric hetero-Diels-Alder reaction was used to prepare a library of dihydropyrancarboxamides in which both substitution and configuration was varied (structures **7** and their enantiomers) (Scheme 1.2).[28] The library of 4320 structures was encoded using a binary encoding protocol which involved the attachment of tags to individual macrobeads. The asymmetric step (**5** → **6**) served a number of purposes: it generated the molecular scaffolds; it introduced some stereochemical diversity; and it incorporated the variable substituent, R^1. Deprotection of the ester, amide formation, and release from solid support gave the final compounds. The library enabled the discovery of haptamide A, a ligand which targeted a subunit of the transcription factor, Hap3p.[28]

1.2.3 Synthesis of Compound Libraries with High Scaffold Diversity

The development of robust and reliable synthetic methods that yield a range of diverse small molecule scaffolds has proved extremely demanding. In this section, we describe the progress that has been made towards developing synthetic strategies that allow the scaffolds of small molecules to be systematically varied: in particular, the development of "folding" pathways (Section 1.2.3.1), "branching" pathways (Section 1.2.3.2) and oligomer-based approaches (Section 1.2.3.3).

1.2.3.1 Use of "Folding Pathways" to Introduce Scaffold Diversity

The "folding pathway" approach uses common reaction conditions to transform a range of substrates into products with alternative molecular scaffolds. The substrates are encoded to "fold" into the alternative scaffolds through strategically placed appending groups (sometimes known as σ-elements[29]). This strategy leads to scaffold diversification under substrate control.

Schreiber has developed a folding pathway which exploits the Achmatowicz reaction (Scheme 1.3).[29] The fate of oxidation of the furan substrates **10**