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Chemical biology approaches targeting the actin cytoskeleton through phenotypic screening

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The actin cytoskeleton is dysregulated in cancer, yet this critical cellular machinery has not translated as a druggable clinical target due to cardio-toxic side-effects. Many actin regulators are also considered undruggable, being structural proteins lacking clear functional sites suitable for targeted drug design. In this review, we discuss opportunities and challenges associated with drugging the actin cytoskeleton through its structural regulators, taking tropomyosins as a target example. In particular, we highlight emerging data acquisition and analysis trends driving phenotypic, imaging-based compound screening. Finally, we consider how the confluence of these trends is now bringing functionally integral machineries such as the actin cytoskeleton, and associated structural regulatory proteins, into an expanded repertoire of druggable targets with previously unexploited clinical potential.

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Introduction

Cytoskeletal deregulation is central to the progression of cancer, with altered behaviors well established in both microtubule and actin systems. Microtubule targeting drugs, such as vincristine and paclitaxel, have long been central to the clinical standard-of-care [1]. In striking contrast, despite its similarly pivotal role, no actin-targeting compounds are currently used in clinical settings. Several well-known compounds directly targeting actin have long been in research use, including phalloidin (1938) [2], cytochalasin D (1969) [3] and latrunculin A (1983) [4]. Failure of clinical translation reflects the fact that these compounds are highly toxic as they do not distinguish between different actin isoforms and, therefore, impact both the heart and diaphragm [5]. Screening of compounds derived from marine organisms, fungi and myxobacteria have identified new compounds that also directly target actin [6–9], yet these too cause cardio-toxicity such that they are incompatible with clinical applications. For these reasons, the therapeutic potential of drug-based actin cytoskeleton-targeting remains untapped.

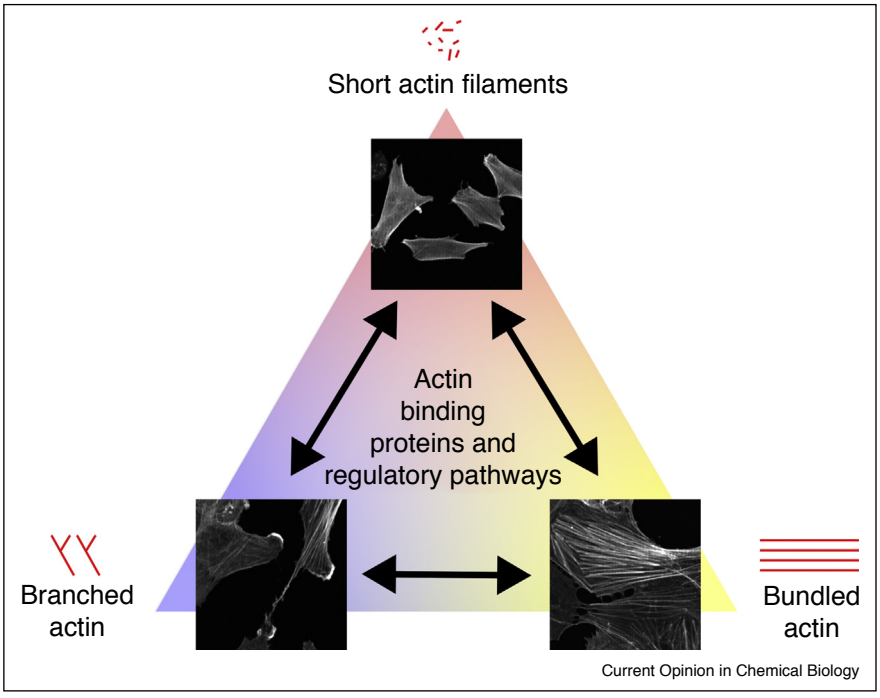
Modulating the actin cytoskeleton through targeting of actin-regulatory networks

Actin-binding and regulatory protein networks provide many opportunities to modulate the actin cytoskeleton, whilst also offering the potential to bypass lethal cardiac toxicity [10]. This is because the tissue-specific, temporal, disease-induced and intracellularly distinct localizations of such regulatory mechanisms may allow for more selective targeting of actin-specific cellular processes (Figure 1). Indeed, the molecular diversity of actin-regulation suggests the existence of numerous 'control points' through which this system may be subtly modulated [11,12]. Only a few are as yet targeted by known compounds.

Beyond enzymes; opportunities and challenges in targeting structural proteins

Potentially druggable actin-regulating proteins can be divided into two broad classes for which drug development strategies differ significantly (Table 1). Enzymatic proteins such as kinases have well-defined substrates and catalytic domains that guide schemas for the design of compound libraries enriched in anti-kinase activity (reviewed in Refs. [13,14•]). Compound efficacy is then readily assessed by narrowly focused measurement of kinase activity, often in high-throughput formats [15]. In contrast to enzymatic targets, structural proteins and complexes such as tropomyosins and Arp2/3 constitute a largely unexploited cohort of potential drug targets, in part because they present additional challenges for drug-discovery. Firstly, they lack unified active sites to be targeted, meaning that new protein structures are often required to enable functional domain mapping and binding pocket identification as precursors to rational drug-design. In the absence of such structural insights, an alternative to targeted drug-design lies in the use of large and diverse compound libraries for unbiased drug-discovery. Here, a second challenge emerges, since readouts of compound activity require assays attuned to functional

Figure 1



The organizational states of actin filaments are regulated via the complex interactions of actin binding proteins and regulatory pathways. Examples of the types of actin filaments that can be generated within a cell are shown in the phalloidin stained images of SK-N-SH neuroblastoma cells that have been treated with compounds that induce each of the 3 different types of actin filaments (branched, short and bundled). Multiple actin filament organizational states can be present within the one cell and this is dependent on the subcellular concentration gradients of individual actin binding proteins and regulatory pathways within the cell (represented by the color gradient). The diversity of actin filament organization seen within cells requires a multi-parametrical approach to image analysis for the quantification of filamentous actin structures.

effects, which in the case of structural proteins may be diverse as well as variable in magnitude [16]. A broad array of measurements is, therefore, beneficial in order to effectively assess and compare compound activity toward structural protein targets. These challenges are here illustrated in the context of drug development targeting tropomyosins, which, as structural proteins regulating F-actin, exemplify the 'expansion of the druggable' to both the actin-cytoskeleton as a biological system, and structural proteins as an incipient class of protein target.

Table 1

Inhibitors of selected enzymes and structural proteins that have an impact on cytoskeletal organization

Enzyme	Rho Kinase (ROCK)	Multiple inhibitors (>170) reviewed in Ref. [49]
	LIM Kinase (LIMK)	Multiple inhibitors (>11 classes) reviewed in Ref. [50]
	PI3 Kinase (PI3K)	Multiple inhibitors in clinical use, reviewed in Ref. [51]
	Myosin Light Chain Kinase (MLCK)	ML-7, ML9 [52]
	Myosin Light Chain Phosphatase (MLCP)	5-Dimethylamino-naphthalene-1-sulfonic acid 4-(2-guanidino-thiazol-4-yl)-phenyl ester [53]
	Focal adhesion kinase (FAK)	Multiple inhibitors, reviewed in Ref. [54]
	Abl kinase (and BcrAbl fusion protein)	Multiple inhibitors in clinical use, reviewed in Ref. [55]
Structural	Arp2/3	CK-666, CK-636, CK-548 [16]
	Tropomyosin 3.1	TR100, ATM1001, ATM3507 [20,21,22]
	Talin	Indothiazinone [56]
	Cofilin	Cucurbitacin E [57]
	Formins	SMIFH2 [58]
	Paxillin	6-B345TTQ [59]
	Fascin	Migrastatin [60]
	N-Wasp	Wiskostatin [61]

Tropomyosins; structural protein targets for selective modulation of the actin cytoskeleton

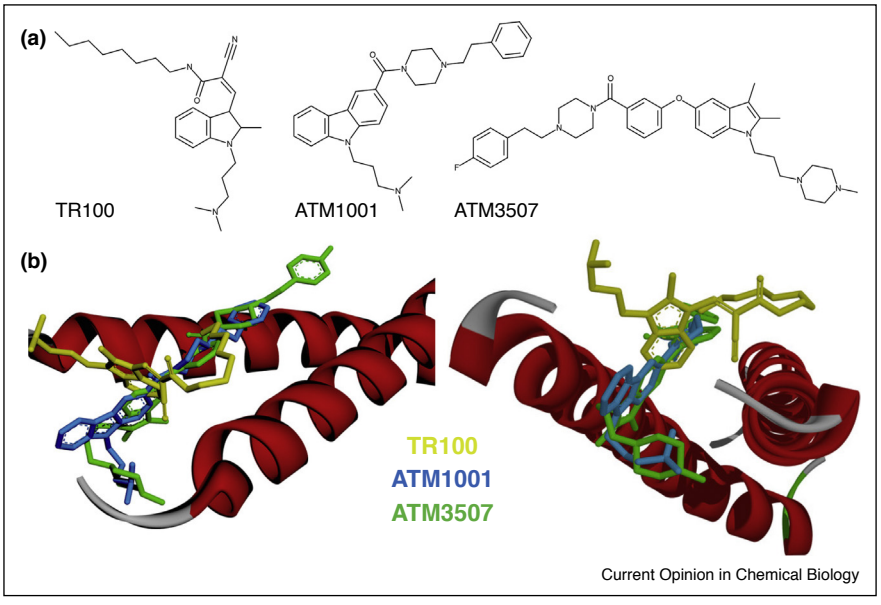
The majority of actin filaments are not naked, but are in fact coated with tropomyosins (Tpm) which play decisive roles in the elaboration of virtually all actin filament structures [17•]. Forming a family of >40 isoforms from 4 genes, these structural proteins are highly regulated during development, displaying distinct spatial and temporal distributions within tissues [18]. Notably, the tropomyosin isoform Tpm3.1 is highly and specifically upregulated in all cancer cell lines tested to-date, thereby constituting a potentially selective target for actin-oriented cancer therapy [17•,19,20]. Indeed, Tpm3.1-targeting has the potential to circumvent problems of cardiac (and other muscle) toxicity that have previously precluded actin cytoskeleton-directed therapies from progressing to the clinic.

Tropomyosins are coiled–coiled proteins that dimerize during actin binding. Accordingly, efforts toward targeted drug-design have focused on regions of overlap at N-terminal and C-terminal, where substantial inter-isoform diversity permits selective targeting strategies. To date, three compounds have been identified with selective anti-Tpm3.1 activity: TR100 [20]; ATM1001 [21], and; ATM3507 [22•] (Figure 2a). Significantly, these compounds are structurally distinct and have different amino acid interactions within the binding pocket, highlighting how – particularly in the context of targeting structural proteins – diverse molecular scaffolds can produce similar biological outcomes (Figure 2b). Notably, these three compounds were discovered via molecular modelling of the C-terminal protein structure only, coupled with similarly localized binding pocket analysis. As a consequence, the druggable potential of the remaining ~90% of the tropomyosin structure remains unexplored.

Targeting isoforms using drug design

Many of the proteins which collaborate with actin to generate functional diversity are members of multi-gene/multi-isoform families and disease specific isoforms have been identified [10]. The ability to target the cancer-associated tropomyosin Tpm3.1 but avoid the muscle tropomyosins highlights the opportunity to target specific protein isoforms [20,21,22•]. A considerable research effort has been directed at targeting the actin isoforms found in cancer cells but the absence of isoform-specific binding pockets and the conservative nature of amino acid substitutions between isoforms have likely contributed to the failure to produce actin isoform-specific drugs [10,23]. In contrast, the Arp2/3 complex appears ideally suited to this approach where substantial variation exists in the ARPC isoforms [24–27]. Furthermore, the existence of a crystal structure of the inhibitor CK666 bound to the Arp2/3 complex is a potential starting point for designing isoform-specific drugs [16]. However, reliance on drug design results in a focus on the more obvious binding pockets and ignores the remainder of the molecule. In order to more fully exploit the extensive structural and chemical space that is beyond the scope of molecular modelling, drug-discovery across the whole

Figure 2



Anti-tropomyosin 3.1 inhibitors. **(a)** The chemical structures of TR100, ATM1001, and ATM3507. **(b)** An overlay of the predicted interaction of the 3 anti-Tpm3.1 compounds with the C-terminus (9d exon) of Tpm3.1.

structure demands the application of alternative strategies.

Drug-discovery targeting structural proteins through phenotypic screening

Targeting of structural proteins requires exploration of a large and diverse chemical space in order to identify compounds with optimal therapeutic properties, including specificity, potency, bio-availability, and kinetics. In many cases, where a lack of structural data precludes rational drug-design, drug-discovery based on screening of large compound libraries provides a credible alternative strategy. In particular, 'phenotypic' screening based on imaging (as opposed to alternative methods of rich cellular data acquisition) [28] enables the measurement of a diversity of effects on subcellular systems and overall cell morphology. Such phenotypic measurements are achieved through computational image analysis enabling extraction of multivariate feature sets quantitatively characterizing classical morphometric features of object (i.e. cell and/or intracellular structure) number, size, shape, intensity, and texture [29], or more abstract features selected through the application of machine-learning approaches (JC Caicedo *et al.*, bioRxiv doi:https://doi.org/10.1101/335216). This facilitates large-scale measurement and comparison of compound effects on complex cellular architectures. For example, in the context of actin-targeting drug-discovery, we have established an imaging-based actin phenotyping workflow that integrates a customized filament detection algorithm with numerous measures of cell and nuclear morphology [30]. This generates multivariate datasets with the capacity to detect and distinguish various subtle effects on actin organization resulting from tens of thousands of chemical perturbations (Figure 3). By quantitatively parsing diverse drug effects, such phenotypic screening allows the identification of compounds impacting particular biological processes [31]. Moreover, compounds with known mechanism-of-action (MOA) can be leveraged, much like landmarks, to predict unknown compound MOA based on similarities in compound-induced phenotypes [32–34]. Objective detection of such similarities is typically automated via quantitative co-clustering and/or statistical distance metric analysis [35••,36].

Extension and enhancement of phenotypic screens

Phenotypic screening can produce a wealth of information, yet each screen constitutes a major investment in time and resources [37]. As such, methods to maximize and even extend the utility of screening data are highly desirable. For instance, no matter their scale, a given drug-discovery screen can contain only a limited number of positive controls, that is, compounds with known MOA. With such positive controls enabling accelerated MOA prediction toward unknown compounds (by virtue of phenotypic similarity), the number of positive controls in a screen is roughly proportional to its overall capacity for both mechanism prediction and lead-compound discovery. One exciting method to overcome this limitation has been demonstrated by showing that phenotypic clustering of known and unknown compounds in a 'generic' imaging-based assay can provide significant predictive capacity – mediated by machine-learning – toward the MOA of the unknown compounds when assessed in a range of separate mechanistic assays [38••].

Another strategy by which to extend and accelerate understanding of unknown compound MOA is to combine chemical screening with analyses of genetic variation, thereby highlighting specific molecular components associated with chemically induced phenotypes. Analogous to chemical genetic studies of growth responses in bacteria and yeast strains with known genetic mutational backgrounds [39,40], this approach has recently been applied through comparison of phenotypic responses in various cell lines with distinct genetic backgrounds [41,42]. A more direct route to this end is also emerging, whereby chemically and genetically (RNAi/CRISPR) induced phenotypes are either compared between datasets [43] or directly parallelized within a single phenotypic screen [32].

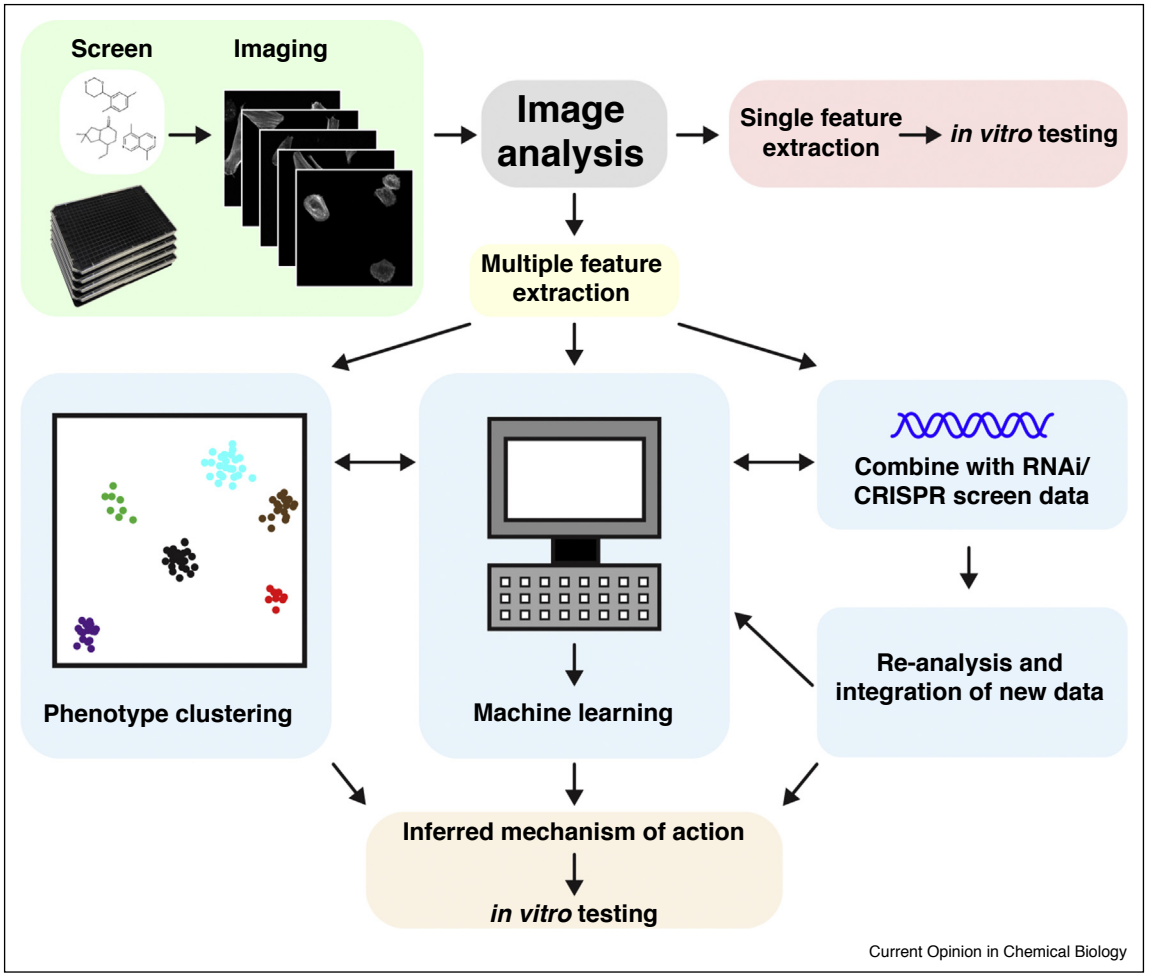
To maximize screen sensitivity and capacity to discern complex phenotypes, increasing the number of molecular or cell-state markers accessed during imaging is hugely valuable, since this is the basis for all phenotypic insights and mechanistic inference. Increasing the marker numbers has been achieved through optical multiplexing (of up to six markers) [44••], and also by synthetic multiplexing strategies linking data containing recurrent 'core' markers – used to define phenotypic states – with additional markers that extend the scope of biological insights [45].

Collectively, these approaches, along with efforts to share [46], annotate [47] and uniformly describe [48] phenotypic screening data, have great potential to amplify the value of such data through its integration and re-use in both drug-discovery and fundamental biological research.

Expanding the druggable; rethinking the proteins and systems amenable to clinical targeting

The emergence, efficacy, and increasing accessibility of phenotypic compound screening methods now enables a new generation of drug discovery efforts targeting structural proteins with selective effects beyond the reach of existing enzymatic targets. The large chemical compound space associated with structural proteins now becomes part of a greatly expanded druggable landscape, and this is true even where *a priori* protein-structure knowledge of potential targets remains lacking. Importantly, the diversity of effects resulting from structural protein modulation provides opportunities for subtle 'tuning' of cellular properties, as opposed to dramatic disruption of more

Figure 3



Alternate analysis pathways of multivariate data allow datasets to be interrogated in different ways to obtain maximal phenotypic data in comparison to the analysis of a single feature.

binary biological processes. This may enable combinatorial therapies with synergistic effects to be devised, maximizing clinical impact whilst minimizing side-effects. Given these exciting advances, practitioners of drug development may consider anew which cellular systems are in fact amenable to clinical intervention. Thus, phenotypic screening approaches are beginning to expand the druggable landscape to new classes of protein, and also to new biological target systems.

Practicalities of phenotypic screening the actin cytoskeleton

The attraction of phenotypic screening of the actin cytoskeleton needs to be balanced by the practicalities which must be confronted in such an approach as follows:

1 Cells used for such a screen must have a highly conserved actin organization between individual cells. For example, the neuroblastoma SK-N-SH cells consistently display a well-organized cytoskeleton composed of actin filament bundles as shown in Figure 1. Too much variation gives rise to a weak signal to noise signal when the cytoskeleton is impacted by drug exposure.
2 Staining and visualization of the actin cytoskeleton need to withstand robotic manipulation. Procedures need to be optimized for robotic liquid handling and not human manipulation that is much milder and less prone to mechanical disruption of the cells.
3 To ensure that multiple different actin phenotypes are detected, the imaging approach to phenotype should include measurement of multiple parameters that include intensity and organization of filaments, cell size and shape and cytoplasmic clustering of actin into clusters. Examples of the variety of actin phenotypes that can occur are shown in Figure 1. We have used both a program designed to measure specific features of actin organization [30] and Columbus software (Perkin

Elmer) which measures more general morphological properties of the cells.

4 Any cytoskeleton drug screen should avoid impacts on contractile cells such as cardiac and skeletal muscle cells. Unlike non-muscle cells, the cytoplasm of cardiac cells is filled with highly specialized actomyosin contractile units. A simple approach to identify compounds that have cardiac sensitivity is to test any 'hits' for impact on cultured cardiomyocytes [20]. In contrast, cancer cells show a range of architectural phenotypes ranging from well-organized bundles of filaments as seen with SK-N-SH neuroblastoma (Figure 1) to diffuse organization with little evidence of filament bundles [17].

Chemical biology of the actin cytoskeleton

At the vanguard of these changes are drug development efforts focusing on the actin cytoskeleton as a target for cancer therapy. Importantly, these efforts now also feed back to support our understanding of the actin cytoskeleton as an integrated biological system. Historically, conventional molecular reductionist approaches relying on piecewise genetic manipulation of known actin filament regulators have served to establish relatively binary principles of actin filament biochemistry. Yet these isolated mechanisms have not combined to illuminate a coherent view of the actin system in its totality. Now, phenotypic screening of diverse chemical libraries allows for unbiased system-wide perturbations that reveal subtle, interdependent and non-linear regulatory relationships within networks of known and previously unknown regulatory actors. As a consequence, perceived edges of the actin regulatory system are blurring, and we begin to elicit more integrated models of actin system regulation and function as a whole. In future, such a chemical biology analyses of the actin cytoskeleton promise to take us beyond our fragmented view of isolated, binary mechanisms to a more comprehensive, coherent, and quantitative understanding of this fundamental cellular system.

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Conflict of interest statement

PWG and ECH are Directors of TroBio Therapeutics, a company that is commercializing anti-tropomyosin drugs for the treatment of cancer and their labs receive funding from TroBio Therapeutics to evaluate anti-tropomyosin drug candidates.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Steinmetz MO, Prota AE: **Microtubule-targeting agents: strategies to hijack the cytoskeleton**. *Trends Cell Biol* 2018, **28**:776-792.

2. Lynen F, Wieland U: **Über die Giftstoffe des Knollenblätterpilzes. IV**. *Justus Liebigs Ann Chem* 1938, **533**:93-117.

3. Aldridge DC, Turner WB: **Structures of cytochalasins C and D**. *J Chem Soc C: Org* 1969:923-928.

4. Spector I, Shochet NR, Kashman Y, Groweiss A: **Latrunculins: novel marine toxins that disrupt microfilament organization in cultured cells**. *Science* 1983, **219**:493-495.

5. Creed SJ, Bryce N, Naumanen P, Weinberger R, Lappalainen P, Stehn J, Gunning P: **Tropomyosin isoforms define distinct microfilament populations with different drug susceptibility**. *Eur J Cell Biol* 2008, **87**:709-720.

6. Foerster F, Braig S, Chen T, Altmann KH, Vollmar AM: **Pharmacological characterization of actin-binding (-)-doliculide**. *Bioorg Med Chem* 2014, **22**:5117-5122.

7. Herrmann J, Hüttel S, Müller R: **Discovery and biological activity of new chondramides from *Chondromyces* sp**. *ChemBioChem* 2013, **14**:1573-1580.

8. Schweikart K, Guo L, Shuler Z, Abrams R, Chiao ET, Kolaja KL, Davis M: **The effects of jaspamide on human cardiomyocyte function and cardiac ion channel activity**. *Toxicol In Vitro* 2013, **27**:745-751.

9. Howarth FC, Boyett MR, White E: **Rapid effects of cytochalasin-D on contraction and intracellular calcium in single rat ventricular myocytes**. *Pflügers Arch* 1998, **436**:804-806.

10. Bonello TT, Stehn JR, Gunning PW: **New approaches to targeting the actin cytoskeleton for chemotherapy**. *Future Med Chem* 2009, **1**:1311-1331.

11. Steffen A, Stradal TE, Rottner K: **signalling pathways controlling cellular actin organization**. *Handb Exp Pharmacol* 2017, **235**:153-178.

12. Pollard TD: **Actin and actin-binding proteins**. *Cold Spring Harb Perspect Biol* 2016, **8**.

13. Ferguson FM, Gray NS: **Kinase inhibitors: the road ahead**. *Nat Rev Drug Discov* 2018, **17**:353-377.

14. • Leroux AE, Schulze JO, Biondi RM: **AGC kinases, mechanisms of regulation and innovative drug development**. *Semin Cancer Biol* 2018, **48**:1-17.
An extremely thorough comparison of drug-design strategies for kinase targeting, highlighting commonalities across this enzyme class.

15. Shen M, Tian S, Pan P, Sun H, Li D, Li Y, Zhou H, Li C, Lee SM-Y, Hou T: **Discovery of Novel ROCK1 inhibitors via integrated virtual screening strategy and bioassays**. *Sci Rep* 2015, **5**:16749.

16. Nolen BJ, Tomasevic N, Russell A, Pierce DW, Jia Z, McCormick CD, Hartman J, Sakowicz R, Pollard TD: **Characterization of two classes of small molecule inhibitors of Arp2/3 complex**. *Nature* 2009, **460**:1031.

17. • Meiring JCM, Bryce NS, Wang Y, Taft MH, Manstein DJ, Liu Lau S, Stear J, Hardeman EC, Gunning PW: **Co-polymers of actin and tropomyosin account for a major fraction of the human actin cytoskeleton**. *Curr Biol* 2018, **28**:2331-2337.e2335.
The authors were the first to biochemically quantitate the total amount of individual tropomyosin isoforms within a cell and calculate that the majority of actin filaments within the cell are indeed coated with tropomyosin.

18. Gunning PW, Hardeman EC, Lappalainen P, Mulvihill DP: **Tropomyosin - master regulator of actin filament function in the cytoskeleton**. *J Cell Sci* 2015, **128**:2965-2974.

19. Coombes JD, Schevzov G, Kan CY, Petti C, Maritz MF, Whittaker S, Mackenzie KL, Gunning PW: **Ras transformation overrides a proliferation defect induced by Tpm3.1 knockout**. *Cell Mol Biol Lett* 2015, **20**:626-646.

20. Stehn JR, Haass NK, Bonello T, Desouza M, Kottyan G, Treutlein H, Zeng J, Nascimento PR, Sequeira VB, Butler TL *et al.*: **A novel class of anticancer compounds targets the actin cytoskeleton in tumor cells**. *Cancer Res* 2013, **73**:5169-5182.

21. Kee AJ, Chagan J, Chan JY, Bryce NS, Lucas CA, Zeng J, Hook J, Treutlein H, Laybutt DR, Stehn JR *et al.*: **On-target action of anti-tropomyosin drugs regulates glucose metabolism**. *Sci Rep* 2018, **8**:4604.

22. • Currier MA, Stehn JR, Swain A, Chen D, Hook J, Eiffe E, Heaton A, Brown D, Nartker BA, Eaves DW *et al.*: **Identification of cancer-targeted tropomyosin inhibitors and their synergy with microtubule drugs**. *Mol Cancer Ther* 2017, **16**:1555-1565.

The authors identify ATM3507 as a small molecule that inhibits Tpm3.1. This study was the first to show synergy between anti-tropomyosin and anti-microtubule compounds on neuroblastoma xenografts *in vivo*.

23. Perrin BJ, Ervasti JM: **The actin gene family: function follows isoform**. *Cytoskeleton (Hoboken)* 2010, **67**:630-634.

24. Abella JV, Galloni C, Pernier J, Barry DJ, Kjaer S, Carlier MF, Way M: **Isoform diversity in the Arp2/3 complex determines actin filament dynamics**. *Nat Cell Biol* 2016, **18**:76-86.

25. Jay P, Berge-Lefranc JL, Massacrier A, Roessler E, Wallis D, Muenke M, Gastaldi M, Taviaux S, Cau P, Berta P: **ARP3beta, the gene encoding a new human actin-related protein, is alternatively spliced and predominantly expressed in brain neuronal cells**. *Eur J Biochem* 2000, **267**:2921-2928.

26. Balasubramanian MK, Feoktistova A, McCollum D, Gould KL: **Fission yeast Sop2p: a novel and evolutionarily conserved protein that interacts with Arp3p and modulates profilin function**. *EMBO J* 1996, **15**:6426-6437.

27. Millard TH, Behrendt B, Launay S, Futterer K, Machesky LM: **Identification and characterisation of a novel human isoform of Arp2/3 complex subunit p16-ARC/ARPC5**. *Cell Motil Cytoskeleton* 2003, **54**:81-90.

28. Feng Y, Mitchison TJ, Bender A, Young DW, Tallarico JA: **Multi-parameter phenotypic profiling: using cellular effects to characterize small-molecule compounds**. *Nat Rev Drug Discov* 2009, **8**:567-578.

29. Eliceiri KW, Berthold MR, Goldberg IG, Ibanez L, Manjunath BS, Martone ME, Murphy RF, Peng H, Plant AL, Roysam B *et al.*: **Biological imaging software tools**. *Nat Methods* 2012, **9**:697-710.

30. Vindin H, Bischof L, Gunning P, Stehn J: **Validation of an algorithm to quantify changes in actin cytoskeletal organization**. *J Biomol Screen* 2014, **19**:354-368.

31. Scheeder C, Heigwer F, Boutros M: **Machine learning and image-based profiling in drug discovery**. *Curr Opin Syst Biol* 2018, **10**:43-52.

32. Caicedo JC, Singh S, Carpenter AE: **Applications in image-based profiling of perturbations**. *Curr Opin Biotechnol* 2016, **39**:134-142.

33. Reisen F, Sauty de Chalon A, Pfeifer M, Zhang X, Gabriel D, Selzer P: **Linking phenotypes and modes of action through high-content screen fingerprints**. *Assay Drug Dev Technol* 2015, **13**:415-427.

34. Perlman ZE, Slack MD, Feng Y, Mitchison TJ, Wu LF, Altschuler SJ: **Multidimensional drug profiling by automated microscopy**. *Science* 2004, **306**:1194-1198.

35. •• Caicedo JC, Cooper S, Heigwer F, Warchal S, Qiu P, Molnar C, Vasilevich AS, Barry JD, Bansal HS, Kraus O *et al.*: **Data-analysis strategies for image-based cell profiling**. *Nat Methods* 2017, **14**:849-863.

An excellent summary of methods used to maximise information and insight from imaging based screens and analyses.

36. Ljosa V, Caie PD, Ter Horst R, Sokolnicki KL, Jenkins EL, Daya S, Roberts ME, Jones TR, Singh S, Genovesio A *et al.*: **Comparison of methods for image-based profiling of cellular morphological responses to small-molecule treatment**. *J Biomol Screen* 2013, **18**:1321-1329.

37. Dorval T, Chanrion B, Cattin M-E, Stephan JP: **Filling the drug discovery gap: is high-content screening the missing link?** *Curr Opin Pharmacol* 2018, **42**:40-45.

38. •• Simm J, Klambauer G, Arany A, Steijaert M, Wegner JK, Gustin E, Chupakhin V, Chong YT, Vialard J, Buijnsters P *et al.*: **Repurposing high-throughput image assays enables biological activity prediction for drug discovery**. *Cell Chem Biol* 2018, **25**:611-618.e613.

The authors use machine learning techniques to repurpose data from a single high throughput assay, developing machine learning-based models to enrich the prediction of compound activity in a range of focused mechanistic screens.

39. Hillenmeyer ME, Fung E, Wildenhain J, Pierce SE, Hoon S, Lee W, Proctor M, St Onge RP, Tyers M, Koller D *et al.*: **The chemical genomic portrait of yeast: uncovering a phenotype for all genes**. *Science* 2008, **320**:362-365.

40. Nichols RJ, Sen S, Choo YJ, Beltrao P, Zietek M, Chaba R, Lee S, Kazmierczak KM, Lee KJ, Wong A *et al.*: **Phenotypic landscape of a bacterial cell**. *Cell* 2011, **144**:143-156.

41. Caie PD, Walls RE, Ingleston-Orme A, Daya S, Houslay T, Eagle R, Roberts ME, Carragher NO: **High-content phenotypic profiling of drug response signatures across distinct cancer cells**. *Mol Cancer Ther* 2010, **9**:1913-1926.

42. Warchal SJ, Dawson JC, Carragher NO: **Development of the theta comparative cell scoring method to quantify diverse phenotypic responses between distinct cell types**. *Assay Drug Dev Technol* 2016, **14**:395-406.

43. Ohnuki S, Oka S, Nogami S, Ohya Y: **High-content, image-based screening for drug targets in yeast**. *PLoS One* 2010, **5**:e10177.

44. •• Rohban MH, Singh S, Wu X, Berthet JB, Bray MA, Shrestha Y, Varelas X, Boehm JS, Carpenter AE: **Systematic morphological profiling of human gene and allele function via cell painting**. *eLife* 2017, **6**.

The authors use cell painting to qualitatively assess the connections between genes using morphological profiling. The experiments revealed novel insights into the relationship between different signalling pathways which were confirmed at the transcriptional level.

45. Loo LH, Lin HJ, Steininger RJ 3rd, Wang Y, Wu LF, Altschuler SJ: **An approach for extensibly profiling the molecular states of cellular subpopulations**. *Nat Methods* 2009, **6**:759-765.

46. Williams E, Moore J, Li SW, Rustici G, Tarkowska A, Chessel A, Leo S, Antal B, Ferguson RK, Sarkans U *et al.*: **The image data resource: a bioimage data integration and publication platform**. *Nat Methods* 2017, **14**:775-781.

47. Ljosa V, Sokolnicki KL, Carpenter AE: **Annotated high-throughput microscopy image sets for validation**. *Nat Methods* 2012, **9**:637.

48. Jupp S, Malone J, Burdett T, Heriche JK, Williams E, Ellenberg J, Parkinson H, Rustici G: **The cellular microscopy phenotype ontology**. *J Biomed Semantics* 2016, **7**:28.

49. Feng Y, LoGrasso PV, Defert O, Li R: **Rho Kinase (ROCK) inhibitors and their therapeutic potential**. *J Med Chem* 2016, **59**:2269-2300.

50. Prunier C, Prudent R, Kapur R, Sadoul K, Lafanechère L: **LIM kinases: cofilin and beyond**. *Oncotarget* 2017, **8**:41749-41763.

51. Mayer IA, Arteaga CL: **The PI3K/AKT pathway as a target for cancer treatment**. *Annu Rev Med* 2016, **67**:11-28.

52. Saitoh M, Ishikawa T, Matsushima S, Naka M, Hidaka H: **Selective inhibition of catalytic activity of smooth muscle myosin light chain kinase**. *J Biol Chem* 1987, **262**:7796-7801.

53. Grindrod S, Suy S, Fallen S, Eto M, Toretsky J, Brown ML: **Effects of a fluorescent myosin light chain phosphatase inhibitor on prostate cancer cells**. *Front Oncol* 2011, **1**:27.

54. Lv P-C, Jiang A-Q, Zhang W-M, Zhu H-L: **FAK inhibitors in cancer, a patent review**. *Expert Opin Ther Patents* 2018, **28**:139-145.

55. Rossari F, Minutolo F, Orciuolo E: **Past, present, and future of Bcr-Abl inhibitors: from chemical development to clinical efficacy**. *J Hematol Oncol* 2018, **11**:84.

56. Yang C, Kwon S, Kim SJ, Jeong M, Park JY, Park D, Hong SJ, Jung JW, Kim C: **Identification of indothiazinone as a natural antiplatelet agent**. *Chem Biol Drug Des* 2017.

57. Nakashima S, Matsuda H, Kurume A, Oda Y, Nakamura S, Yamashita M, Yoshikawa M: **Cucurbitacin E as a new inhibitor of cofilin phosphorylation in human leukemia U937 cells**. *Bioorg Med Chem Lett* 2010, **20**:2994-2997.

58. Rizvi SA, Neidt EM, Cui J, Feiger Z, Skau CT, Gardel ML, Kozmin SA, Kovar DR: **Identification and characterization of a small molecule inhibitor of formin-mediated actin assembly**. *Chem Biol* 2009, **16**:1158-1168.

59. Kummer C, Petrich BG, Rose DM, Ginsberg MH: **A small molecule that inhibits the interaction of paxillin and alpha 4 integrin inhibits accumulation of mononuclear leukocytes at a site of inflammation**. *J Biol Chem* 2010, **285**:9462-9469.

60. Chen L, Yang S, Jakoncic J, Zhang JJ, Huang XY: **Migrastatin analogues target fascin to block tumour metastasis**. *Nature* 2010, **464**:1062-1066.

61. Peterson JR, Bickford LC, Morgan D, Kim AS, Ouerfelli O, Kirschner MW, Rosen MK: **Chemical inhibition of N-WASP by stabilization of a native autoinhibited conformation**. *Nat Struct Mol Biol* 2004, **11**:747-755.