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Spotlight

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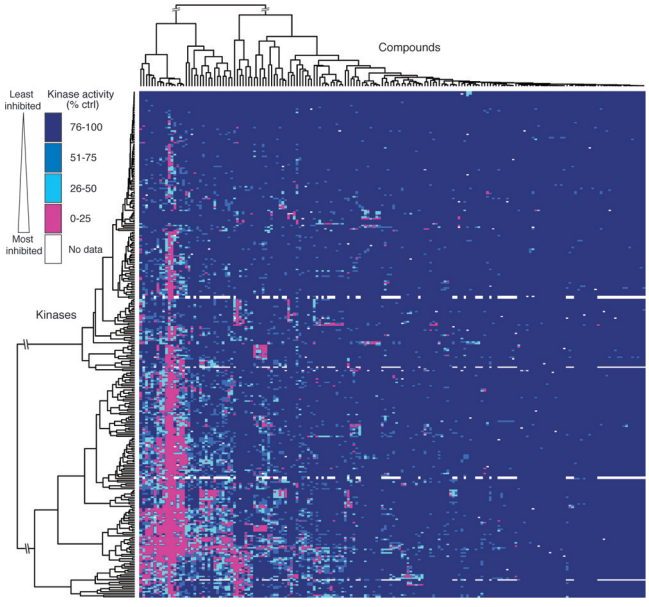
PROFILING FUNCTIONAL INHIBITION

The 518 protein kinases in the human kinome are a cornerstone of the cellular signaling machinery and represent a fruitful and continually promising class of drug targets for numerous diseases. Small molecules not only possess impressive therapeutic prowess against these targets, they are also incredibly powerful molecular tools for deciphering kinase function in various biological contexts. However, the highly conserved ATP-binding pocket of protein kinases, which is the site most commonly targeted by inhibitors, has presented a formidable challenge for identifying selective inhibitors. Moreover, many methods for assessing kinase-inhibitor interactions use binding assays, which do not directly report on inhibition of kinase activity. Now, Anastassiadis *et al.* (*Nat. Biotechnol.* 2011, *29*, 1039−1045) present functional inhibition profiling of a panel of 300 protein kinases by 178 small molecule kinase inhibitors.



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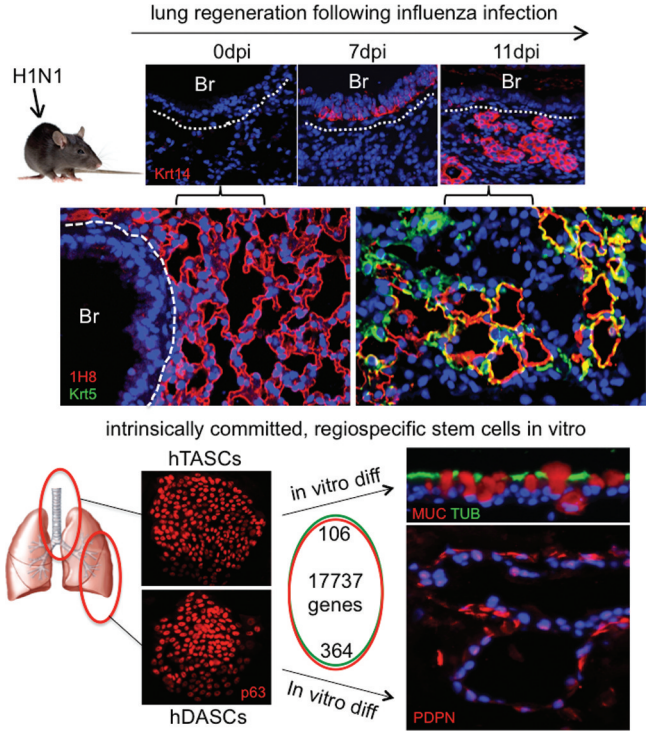
The authors use a radiometric assay that directly measures kinase activity to evaluate the inhibitors against the kinase panel. Two-way hierarchical clustering analysis grouped structurally related compounds, as well as kinases with related sequences, together. However, a few exceptions were identified, illuminating kinases that could potentially be targeted selectively. The compounds were also ranked from the most promiscuous to the most selective, and physicochemical analysis revealed that no single physical parameter is linearly correlated with promiscuity. In addition, compounds were identified that unexpectedly inhibited kinases from diverse classes, highlighting the potential of this type of profiling for the discovery of inhibitors that target multiple distinct therapeutic targets. Analysis for highly selective inhibitors identified 19 compounds that inhibited their primary target at least 20% more potently than other kinases, but surprisingly, 6 compounds were found to inhibit other kinases more potently than their known target. This impressive compilation of data is a tremendous resource for deciphering kinase function, developing new kinase-targeting drugs, and identifying novel therapeutic strategies.

Eva J. Gordon, Ph.D.

PULMONARY PODS

H1N1, the notorious subtype of influenza virus A responsible for killing 40 million people worldwide in 1918 and for the 2009 pandemic that spread to over 200 countries and killed nearly 20,000 people, causes a respiratory infection that can lead to a life-threatening lung condition called acute respiratory distress syndrome (ARDS). ARDS is characterized by excess fluid in the lungs that prevents sufficient levels of oxygen to reach the bloodstream and vital organs. While the destructive path and consequences of ARDS are fairly well-studied, the pathways involved in recovery from ARDS are not well understood. Now, Kumar *et al.* (*Cell* 2011, *147*, 525−538) identify and characterize stem cells that play a key role in the regeneration of lung tissue after ARDS.



Reprinted from *Cell*, *147*, Kumar, P. A., *et al.*, Distal Airway Stem Cells Yield Alveoli In Vitro and during Lung Regeneration following H1N1 Influenza Infection, 525-538. Copyright 2011, with permission from Elsevier.

Published: December 16, 2011

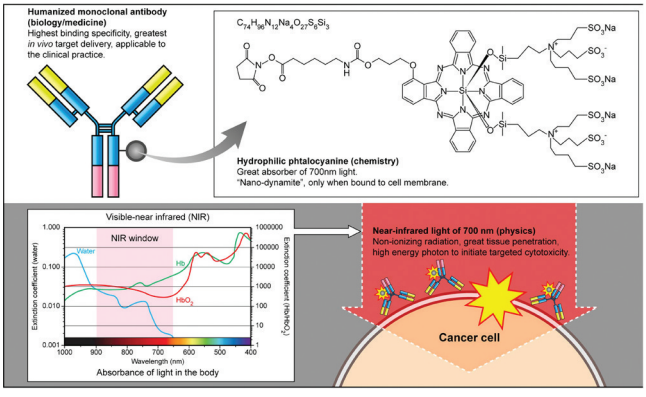
   dx.doi.org/10.1021/cb200477k | *ACS Chem. Biol.* 2011, 6, 1303−1306

Bronchioalveolar stem cells are thought to be progenitor cells for both the bronchioles, the small branching airways in the lungs, and the alveoli, the tiny air sacs at the end of the bronchioles, but the defining characteristics of these cells and their mechanisms of differentiation are unclear. The authors show that in mice infected with H1N1, bronchial-derived cells expressing p63, a transcription factor expressed in stem cells that reside in the nose and windpipe, undergo rapid growth and migrate to regions where alveoli were damaged or destroyed. Once there, they form discrete clusters, referred to as Krt5 pods due to their expression of the structural protein keratin 5, and begin a gene expression program directed toward alveolar and blood vessel generation. *In vitro* studies using a three-dimensional Matrix model that enables investigation of cell differentiation in airways further demonstrated that stem cells derived from distal airway tissues like bronchioles and alveoli have the capacity to generate alveoli-like structures. The intricate pathways involved in lung regeneration illuminated in this study expand our understanding of lung regenerative biology and suggest new therapeutic approaches for lung disease.

Eva J. Gordon, Ph.D.

PITTING PHOTOSENSITIZERS AGAINST CANCER

One of the challenges associated with use of chemotherapy agents for cancer treatment is minimizing the side effects caused by the drugs. Targeted therapies, such as antibodies that inactivate proteins expressed at higher levels on cancer cells than on normal cells, have found some success in surmounting this challenge but still often suffer from dose-limiting toxicities. Conventional photodynamic therapy, in which cells are killed upon activation of a photosensitizing agent with nonionizing light, has also found limited success as a therapeutic strategy for cancer and other conditions. Now, Mitsunaga *et al.* (*Nat. Med.* advance online publication November 6, 2011; DOI: 10.1038/ nm.2554) combine the selectivity of targeted therapy and the potential of phototherapy in the creation of novel photoimmunotherapy (PIT) reagents composed of antibodies conjugated to the photosensitizer IRDye 700DX (IR700).



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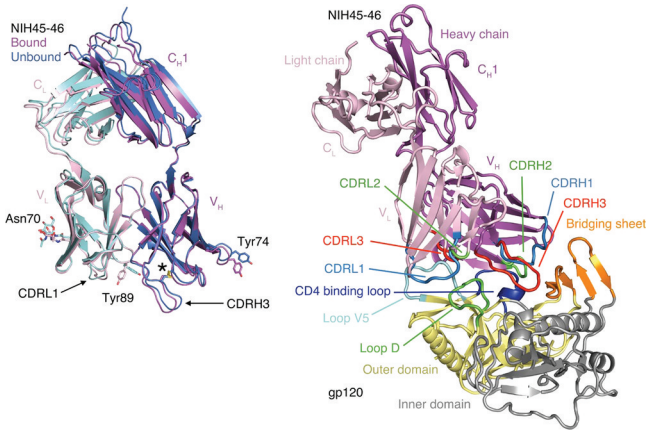
IR700, a near-infrared phthalocyanine dye, was conjugated to a monoclonal antibody to one of two receptors known to play significant roles in cancer: trastuzumab, which targets epidermal growth factor receptor 2, or panitumumab, which binds epidermal growth factor receptor 1. Characterization of the conjugates indicated approximately three IR700 molecules were attached to each antibody. The natural fluorescence of IR700 was exploited to monitor binding of the conjugates to cells, which occurred initially on the cell surface and inside the cells after prolonged incubation. Upon excitation with light, only cells expressing one of the receptors and exposed to the PIT reagent underwent necrotic cell death. In mouse xenograft tumor models, intravenous injection of the conjugate followed by excitation with near-infrared light resulted in tumor shrinkage and enhanced survival. The reagents exhibited no adverse effects up to 8 weeks after treatment. These initial results support the potential of PIT reagents as selective, nontoxic therapeutic agents for cancer and other diseases.

Eva J. Gordon, Ph.D.

OPTIMIZING ON NEUTRALIZING

One of the latest tactics in the war on AIDS is to peer into the immune response of HIV-infected individuals to observe the repertoire of antibodies directed against this deadly virus. Some individuals develop broadly neutralizing antibodies (bNAbs), which can inhibit the infectivity of many known HIV isolates. In the normal HIV life cycle, viral particles are targeted to the immune system by a direct interaction between an HIV envelope protein and a receptor, CD4, expressed on some T-cells. Many recently characterized bNAbs mimic the shape of CD4 and dock with the HIV envelope protein gp120, thus preventing the virus from binding to a cell. With the recent cloning of numerous bNAbs, structural biology has helped to define the precise atomic interactions between antibody polypeptide chains and gp120. Now, a new study from Diskin *et al.* (*Science* advance online publication October 27, 2001; DOI: 10.1126/science.1213782) makes use of structural data to go one step further and rationally design an antibody with improved binding properties.



From Diskin, R., et al., *Science*, **2011**, Advance online publication, DOI: 10.1126/science.1213782. Reprinted with permission from AAAS.

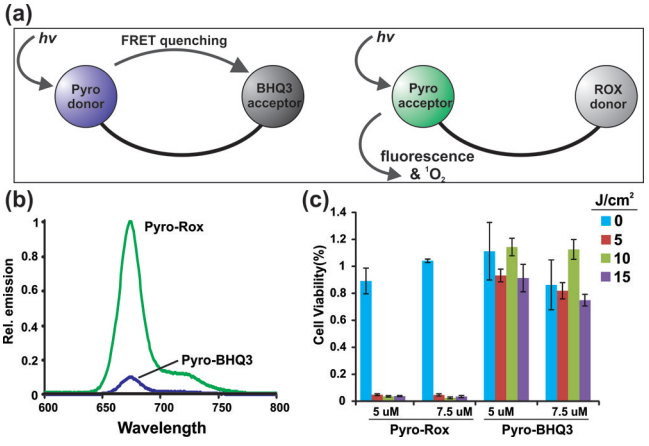
The authors solved the X-ray crystal structure of NIH45-46, a highly potent and broad spectrum antibody, alone or in complex with a fragment of the gp120 protein. One notable difference with this particular antibody was a 4 amino acid insertion and the structure indicated that these residues make specific contacts with side-chains of gp120. Since the antibody acts as a mimic of CD4, the authors also compared the results

to a structure of CD4 with gp120 and noticed an interesting difference. The gp120 protein forms a hydrophobic pocket between the domain known as the bridging sheet and the outer domain. CD4 binding takes advantage of that hydrophobic pocket by burying a phenylalanine side-chain into that space. With this observation in hand, the authors tested several designed mutants of the NIH45-46 antibody to place a hydrophobic side-chain in the gp120 binding pocket. The winner among the mutants, G54W, showed remarkably broad potency in HIV strain neutralization assays. In fact, some strains that were not neutralized by NIH45-46 were neutralized with this single mutation. This study displays the elegant advantage of comparing multiple structures to learn how to optimize a molecular interaction.

Jason G. Underwood, Ph.D.

SPURRING AND MONITORING APOPTOSIS

Photodynamic therapy harnesses light, photosensitizing molecules, and oxygen, to produce singlet oxygen that destroys diseased cells. Challenges remain both in monitoring the treatment dose and in avoiding damage to healthy tissue. One method for following treatment uses molecular strategies that link the photosensitizers to cleavable fluorescent probes. The covalent linkage takes advantage of Förster Resonance Energy Transfer (FRET) and quenches the activity of both the photosensitizer and the probe, but when cleaved in response to apoptotic factors such as caspases, they produce a fluorescent signal.



Reprinted with permission from Lovell, J. F., et al, *J. Am. Chem. Soc.*, **2011**, *133*, 18580−18582. Copyright 2011 American Chemical Society.

Although a useful first step, such systems, known as activatable photosensitizers (aPS), have a paradoxical design flaw. The caspases, which cleave and activate these molecules, require some initial exposure to singlet oxygen, which only occurs after the photosensitizer has been activated *via* protease cleavage and exposed to light. Now Lovell *et al.* (*J. Am. Chem. Soc.* 2011, *133*, 18580−18582) report a new class of these systems, quantifiable, unquenched activatable photosensitizers (QUaPS) that take advantage of a porphyrin photosensitizer that is unquenched while attached to the reporter probe.

The researchers attached the porphyrin pyropheophorbide (Pyro), the photosensitizer, to a 9 amino acid peptide sequence that was specific for caspase-3. The C-terminal residue of that sequence is lysine, and the fluorophore, 5-carboxy-X-rhodamine (Rox), was linked at the epsilon-nitrogen on the amino acid side chain. The absorption spectrum of Pyro overlapped the emission spectrum of Rox, and the FRET radius was greater than 50 Å. Therefore, while the two were attached *via* the linker, excitation of Rox was transferred *via* FRET and led to emission of Pyro at 680 nm. After caspase had cleaved the linker, excitation of Rox led to emission of Rox at 610 nm.

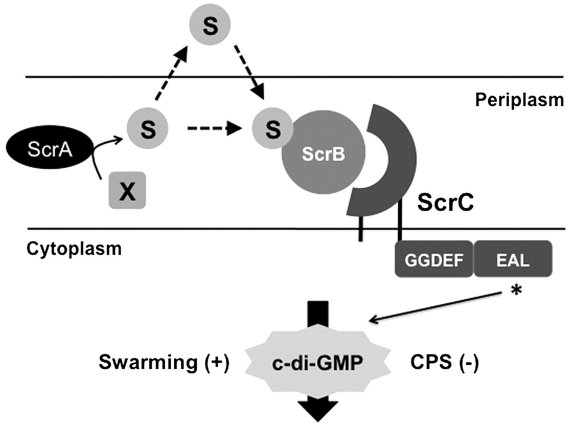
Lovell et al compared this new sensitizer-reporter system with an earlier aPS. Pyro proved to be a more potent photosynthesizer, and HT-29 colorectal cancer cells were efficiently killed over a range of intensities of light. Studies with recombinant caspase-3 and a caspase inhibitor demonstrated that the response was measurable and caspase-dependent.

Overall the work presents a simple approach for producing singlet oxygen and monitoring the apoptotic response of a cell. Because of the limits of light penetration, photodynamic therapy is currently limited to regions close to the surface of skin or tissues, but further development of longer-wavelength photosensitizers could extend the reach of this treatment to tissues deeper within body.

Sarah A. Webb, Ph.D.

NEW BACTERIAL LANGUAGE

Bacteria can synchronize their gene expression in response to population density, a process known as quorum sensing. This behavior is advantageous to the bacteria as it permits them to coordinate survival strategies such as surface adhesion and colonization behaviors. Bacterial cells communicate information about population density through small chemical molecules such as *N*-acyl-homoserine lactones, cyclic peptides, and γ-quinolones. Curiously, pathogenic *Vibrio parahemolyticus* is able to perform typical quorum sensing-related functions such as surface swarming and biofilm formation despite a disrupted quorum sensing pathway. Trimble and McCarter (*Proc. Natl. Acad. Sci. U.S.A.* 2011, *108*, 18079−18084) have solved this conundrum and report a new chemical communication signal that facilitates *V. parahemolyticus* surface swarming.



Trimble, M. J., et al., *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 18079−18084. Copyright 2011 National Academy of Sciences, U.S.A.

It was previously reported that secondary messenger, bis-(3′-5′)-cyclic dimeric GMP (c-di-GMP) seems to affect many quorum-sensing related functions in *V. parahemolyticus*. This observation raised the intriguing possibility that this ubiquitous messenger may in some way be important in a previously

dx.doi.org/10.1021/cb200477k | *ACS Chem. Biol.* 2011, 6, 1303−1306

undiscovered quorum sensing mechanism. The authors focused their efforts on the *scrABC* operon since it is upregulated with growth on surfaces. Additionally, ScrC has been previously reported to modulate c-di-GMP levels. In this study, ScrA, a pyridoxal phosphate-dependent aminotransferase, was shown to produce a signal molecule that was isolated from cell free extracts. This "S-signal" was shown to induce swarming even at low cell densities. Detection of the S-signal is accomplished by the periplasmic-binding receptor, ScrB, and ScrC, the membrane-bound bifunctional protein. Increase in signal causes increase in ScrC phosphodiesterase activity that degrades c-di-GMP resulting in increase in surface swarming. Additionally, this signal repressed capsular polysaccharide biosynthesis genes expression. Thus, the identification of the new quorum-sensing signal and its biosynthetic enzyme represents an expansion in the known language of bacterial cell-to-cell communication.

Jitesh A. Soares, Ph.D.