Click Chemistry: The Certainty of Chance

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If one is fortunate, one can spend time and effort trying to figure out how the world works. Since my college days, I have been obsessed with trying to understand the properties and relationships of the elements. Focusing on Selenium chemistry early in my career,[1] I quickly learned that exciting new reactivity can be found almost anywhere in the Periodic Table, among main group elements (Se, S) as well as transition metals (Ti, Os, Cu) (Figure 1).[2–5] All these years later, I am still obsessed and still learning.

George S. Hammond's profound Norris Award lecture (1968) taught us that "The most *fundamental and lasting objective of synthesis* is not the production of new compounds, but *production of properties*." This resonated with me from the beginning of my career and has guided my research ever since. It is my lifelong mission to provide chemists everywhere with easy access to more power, more speed, more reliability. I have also always regarded simplicity and utility as being more appealing than "elegant" complexity, making me, in essence, a process chemist. In this long hunt for good reactions and interesting reactivity, which began at MIT in the 1970s, my idea was to go fishing in the Table with the help of some fearless colleagues. And so, over the years, beyond the common fare, several unknown "creatures" emerged before us, and some of these strangers even turned out to be keepers!

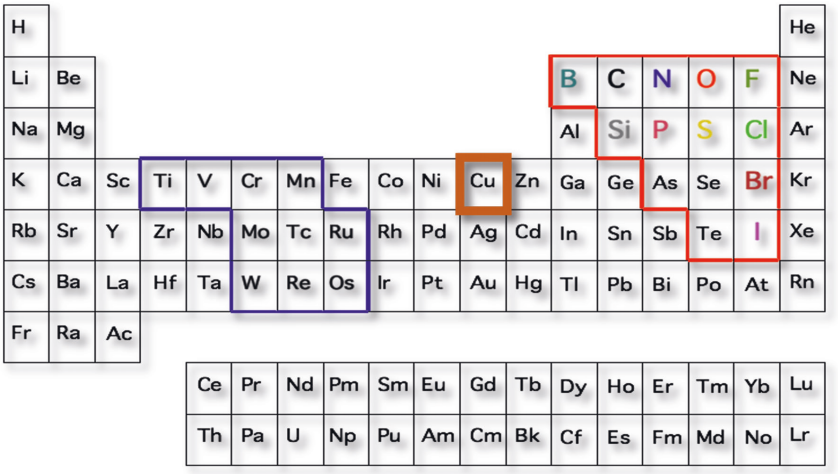


Figure 1. The click chemist's playground.

I started out seeking general solutions to known goals – expanding selectivity in the construction of molecules – but realized in late 1996 that asymmetric catalytic synthesis had become quite unsatisfying for me. Therefore, I decided to act on my core belief that *the best way to achieve powerful (functional) chemistry is through reliable methods of bond formation*. Thus, it became my mission to provide the bond forming tools that would enable the discovery and production of new properties through *process-chemistry driven discovery*. We needed a memorable name for this new way of thinking, and after some consideration, my wife Jan came up with the term "Click Chemistry".

There is so incredibly much to discover, and chemical space is big, really big! The estimable Derek Lowe in 2014 reflected on the "Enumeration of 166 Billion Organic Small Molecules in the Chemical Universe Database GDB-17," by J-L Reymond, U of Bern:[6] "*The best guess for the number of plausible compounds up to molecular weight 500...is around* 10^{60} *...a number that the human mind is not well equipped to handle. That collection, assembled into compound vials at, say, 10 mg per vial, would exceed the amount of ordinary matter in the entire universe.*" Consequently, given the tremendous size of the chemical universe and the countless opportunities within it, we felt that there was no time to waste with complex, multi-step syntheses. Instead, we were convinced from the beginning that click chemistry is the best method, because the better we can rapidly build molecules, the more efficient the exploration of diverse chemical space to find or improve function will be. Hartmuth C. Kolb, M.G. Finn and I laid out our vision in our 2001 Click Chemistry manifesto entitled "Diverse Chemical Function from a Few Good Reactions".[7] Fast and reliable inter-molecular connections are the key. If you have them, you are best

equipped to access the vast chemical space and take advantage of "the certainty of chance."

What do I mean by "the certainty of chance"? The obituary of writer and jazzman George Melly (The Economist, July 12, 2007) illustrates this very well:[8] "*But Mr Melly liked fishing for another reason. As a lifelong Surrealist, he was sure that the bizarre and marvelous lay in wait for him everywhere, and carried in his head a Surrealist motto, 'the certainty of chance'. Chance might give him a fish with the next cast...*" In the unimaginably large sea that is chemical space, then, improving the rod and reel seemed a useful pursuit.

Originally – before the discovery of any perfect reactions – we took our cues from Nature, and her preference for making carbon-heteroatom bonds over carbon-carbon bonds to create her premier functional molecules (Figure 2). We therefore started by exploring spring-loaded reactions that made C-O, C-N, and C-S bonds, finding in the literature many that are "modular, wide in scope, give very high yields, generate only inoffensive byproducts that can be removed by nonchromatographic methods."[7] Later, we added that they should work in (or on) water, since on this planet water and dioxygen are king! Uncannily, the best click chemistry reactions tend to thrive in this terrestrial milieu (Figure 3).[9]

Right after I defined our process-chemistry driven mission back in 1997, which was based on some early "neat chemistry" experiments at Scripps in 1996 by postdoctoral coworker Elizabeth Pease, my lab at Scripps and Hartmuth's group at Coelacanth Corporation got to work. We were a great team: while my lab focused on looking for reliable bond-forming reactions, Hartmuth's lab worked on using them to produce libraries of "drug-like" building blocks and chemical libraries for pharma companies. Initially, Coe-

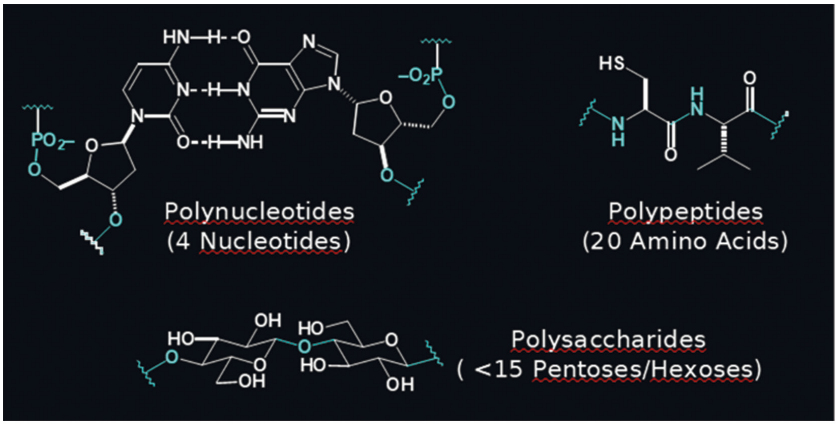


Figure 2. Nature is the original combinatorial chemist. She achieves an immense diversity with < 40 building blocks; large diversity requires 'big molecules'.

Figure 3. The best click chemistry reactions actually thrive in/on/under water.[9]

lacanth was a nascent company, built inside an abandoned Mack truck factory (complete with pigeons and mice!) (Figure 4), so Hartmuth spent his first months with his laptop at a Starbucks in New Brunswick, NJ, to assemble these compounds virtually. Later, we made gram quantities in the lab, exactly as planned, which validated our strong belief that we were on the right track with this approach (Figure 5). The heavy lifting was done by notable Coelacanth chemists, Paul Richardson, David Boulton, Laxma Reddy-Kolla, Zhi-Min Wang, Zhi-Cai Shi, Jay Chiang, Koenraad Vanhessche, Alex Gontcharov, Michael Voronkov, Ram Kanamarlapudi, Ashok Rao Tunoori, Cullen Cavallaro, and many others.

In 1999, Hartmuth and I presented our adventures at the 217th ACS national meeting with a talk entitled "*Click Chemistry: A Concept for Merging Process and Discovery Chemistry*."[10] This was followed by countless presentations around the globe. The Huisgen 1,3-dipolar cycloaddition of azides and alkynes to form disubstituted 1,2,3-triazoles played a prominent role already back then, well before its copper-catalyzed variant (Copper-catalyzed Azide Alkyne Cycloaddition, or CuAAC) emerged (cf. Figure 6). Initially, our views were not met with enthusiasm, and we were accused of just repurposing "old" reactions. What many chemists did not fully grasp back then was that the "old" reactions were often the "best" reactions, and there was every reason to keep exploring and using them. Hartmuth and I started working on our click chemistry manifesto around that time, but it took the masterful touch of M.G. Finn to clearly articulate the concepts.

Click Chemistry wouldn't have happened without the support of Alfred

Figure 4. *Click chemistry lab at Coelacanth* Corporation in New Brunswick, NJ in 1997.

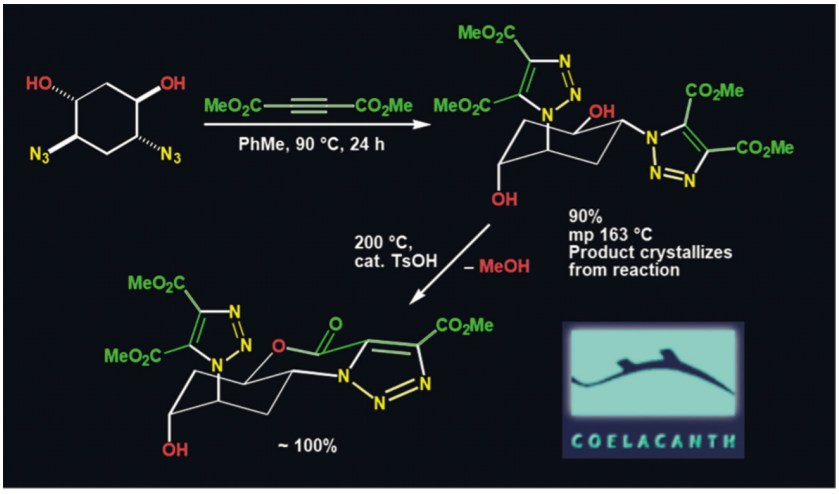


Figure 5. Click Chemistry at Coelacanth – "Rapid Assembly of Drug-Like Molecules".

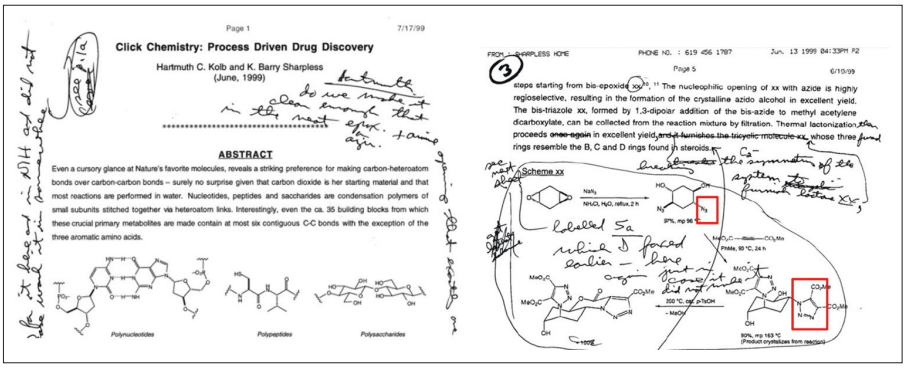


Figure 6. An early draft of our Click Chemistry manifesto (1999). The azide-alkyne Huisgen cycloaddition already played a prominent role.

Figure 7. The people who helped launch click chemistry.

& Isabel Bader and Richard & Nicky Lerner (Figure 7). The late Alfred Bader, the original "Chemist Collector" and Founder of the Aldrich Chemical Company, was not only an angel investor in Coelacanth, but he continued to travel around the world to do what he so much loved doing: collect rare chemicals, which he then provided to us.[11] The late Richard Lerner, then President of Scripps, provided me with all the support I needed to keep my group running and to search for better reactions.[12]

In the late 1990s, M.G. Finn moved to Scripps Research and became the 3rd founding click chemist, and soon thereafter the three of us published our "click manifesto".[7] Recently, the three of us joined forces again to summarize highlights of 20 years of click chemistry.[13]

How is this related to the "Certainty of Chance"? Nature herself is a master in utilizing the certainty of chance for developing properties, a lesson that was beautifully conveyed in Kevin Kelly's book "Out of Control". Here, the author explains that when the simple elements of complex systems (e.g., beehives, cells, immune systems) interact, their *functions* change.[14] Such systems are *Out of [Our] Control* – they are adapting but can't be directed or predicted (Figure 8). Kelly's key message to us was,

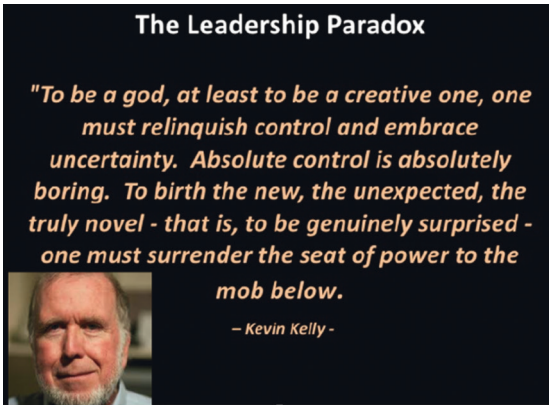
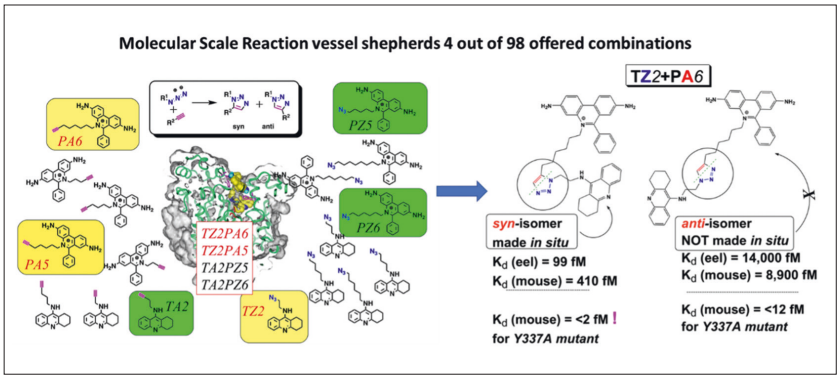


Figure 8. "Out of Control",[14] by Kevin Kelly – The leadership paradox.

"*There's nothing more addictive than being a god. The great irony of god games is that letting go is the only way to win.*"

M.G. and I both read "Out of Control" in December 1999. Within days we had walked the beach discussing it, and ultimately decided to try to adapt the idea to the difficult chemical challenge of creating a potent enzyme inhibitor, but without pre-design. Instead, we presented the enzyme acetylcholinesterase with a variety of azide/alkyne combinations and allowed the target to serve as a molecular-scale reaction vessel for producing its own potent inhibitor, giving birth to "*In Situ Click Chemistry – Enzyme Inhibitors Made to Their Own Specifications*" (Figure 9).[15–17] This was the first indication that we were on the right track! It also illustrated a characteristic property of the boundary-crossing nature of click-enabled science, which was to bring us together with wonderful colleagues in a different discipline. In this case, Palmer Taylor (UCSan Diego pharmacologist) and his team were instrumental in figuring out what the enzyme did, and how.

In situ click chemistry has found numerous applications, such as for the generation of molecular imaging tracers in Hartmuth's lab,[18] and for



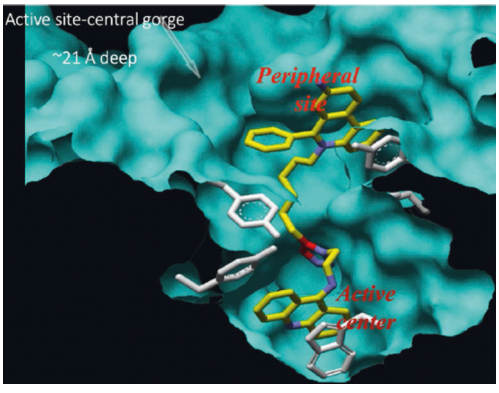


Figure 9. In situ click chemistry with acetylcholinesterase. The enzyme assembles its own inhibitors within its binding sites.[15–17]

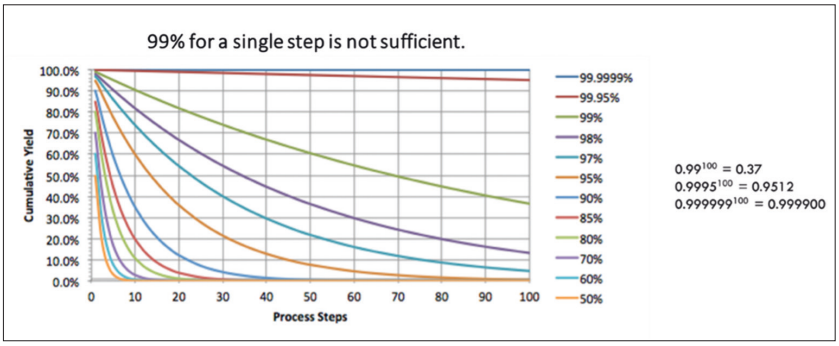


Figure 10. In order for sequential reactions to provide high yields, each individual step must have yields well over 99.9%.

the identification of peptide-based affinity agents (protein-catalyzed capture agents, PCCs) in Jim Heath's lab through the use of single-generation in situ click chemistry screens against large peptide libraries.[19] It has also been performed for screening a large number of azide/alkyne combinations in a micro-fluidics based "lab on a chip."[20]

When we wrote our Click Chemistry manifesto, our early dreams for click chemistry quickly ran into difficulty: not even the best reactions known in 2001 were good enough for our module connection steps!! Until one or more near *perfect* reactions were available, the idea of using even just a few sequential linkup steps with diverse building blocks was not realistic, since one quickly arrives at chaos in any serial linkage scenario if the intermolecular linking reactions are not very close to being perfect. In fact, if the average yield per step is "only" 99% you will have created a mess in 5 or 6 steps (Figure 10). At that time, the only exception was the thiol-ene polymer reaction from Charlie Hoyle's laboratory,[21] which I learned about in a chance encounter with Craig Hawker at the inaugural Cornforth Symposium in 2002 in Sydney, Australia, and which was later named a click reaction. It is the basis of Oleplex for hair care created by Hawker, a striking example of commercial success enabled by reliable chemical bond formation.

In the *in situ* click chemistry by acetylcholinesterase, the triazole made by the enzyme turned out to be the pure *syn* isomer, whereas the thermal Huisgen azide-alkyne cycloaddition made both 1,4 (*anti*) and 1,5 (*syn*) structures. This inspired Luke Green in my lab to try a few metal catalysts, mostly those known to interact well with terminal alkynes. Copper was loud and clear the winner, and I remember being floored by Luke's report to me in the lab the next day, describing the quick completion of a reaction that ordinarily was almost nonexistent at room temperature. This was the birth of the CuAAC process, which was independently discovered by Meldal and Tørnoe in Denmark.[22,23]

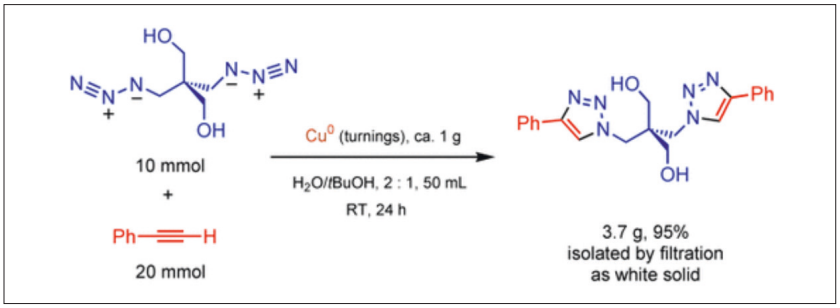


Figure 11. Our first CuAAC publication in 2002: *"By simply stirring in water, organic azides and terminal alkynes are readily and cleanly converted into 1,4-disubstituted 1,2,3-triazoles through a highly efficient and regioselective copper(I)-catalyzed process."*

Looking back, I do believe that our process-chemistry driven way of thinking gave us the tools and necessary focus to go and find – as well as identify and name – such perfect reactions for the first time. My two favorites – the CuAAC (2002, Figure 11)[22] and SuFEx (2014, described below)[24] processes – share a remarkable property: when performed iteratively in a linear stepwise sequence, say 100 times, the overall yield is often close to quantitative! This brought us full circle, back to polymers as an original inspiration for click chemistry: one cannot cleanly make a polymer without extraordinary fidelity and activity in the polymerization reaction (which is why there are so few of them). So, when I met Craig Hawker at the Cornforth Symposium in 2002, sparks flew for both of us! As a world leader in polymer chemistry, Craig was instrumental in driving the adoption of click chemistry by the materials science and polymer communities virtually overnight.[25] A key contribution was our collaboration with the Hawker lab to use the CuAAC process to prepare diverse triazole dendrimers in almost quantitative yield (Figure 12).[26]

Thus, experiencing CuAAC for a few years served as a key point in our own journey to recognizing what a "perfect" reaction was like, and what it could do. Some of the key characteristics are:

- Forging of inter-molecular connections with 99.9+% yields in highly specific fashion, producing only one product/isomer;
- high driving force;
- works on Earth (in the presence of water and O_2); and
- "always works" (other functional groups don't interfere).

Ironically, *the most important certainty-of-chance outcome of click chemistry was our realization that perfect reactions can exist*! Chemistry is quintessentially about bond-making and bond-breaking reactions between atoms and molecules. So, the emergence of "perfect reaction" status promises to

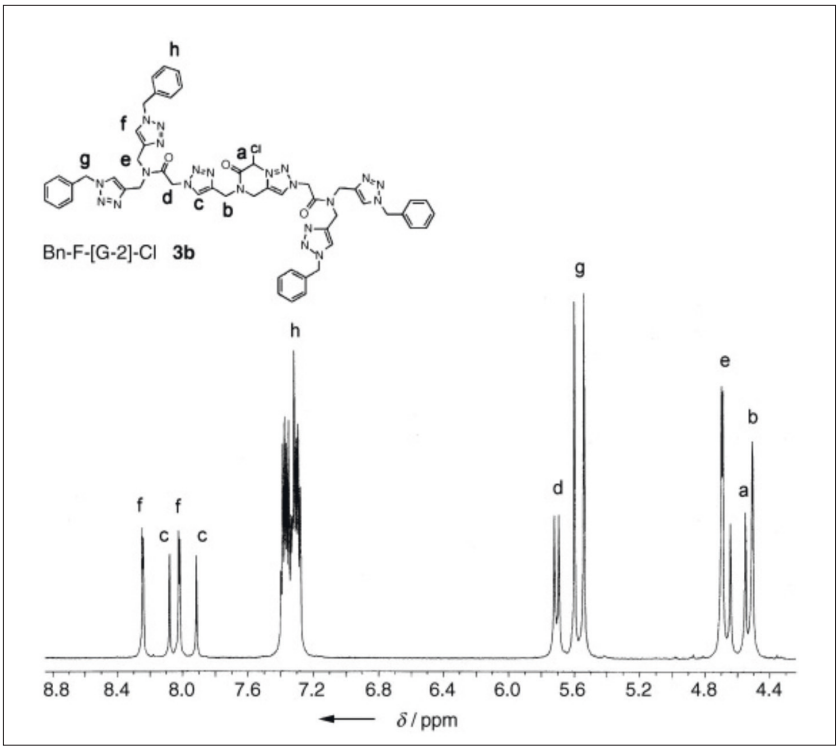
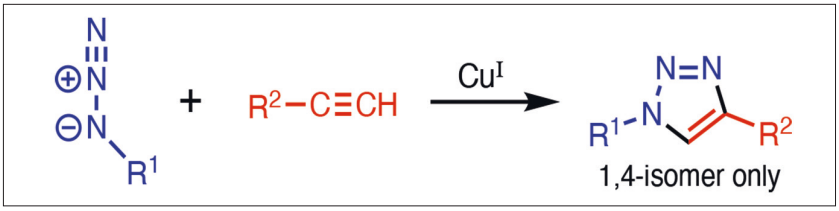


Figure 12. Triazole dendrimers prepared in almost quantitative yield using the CuAAC process.[26]

be transformative to the very heart of chemistry, and thence to the range of benefits for mankind that its future evolution may hold. There are only a few at the moment, but there must be more out there, and now that we know what they look like, they will surely be easier to find.

The CuAAC reaction was a breakthrough success. I consider it a "magic forge" which hammers in triazole links anytime, anyplace, anywhere. Its two key features have made it almost synonymous with the term click chemistry: the azide and alkyne reactive groups are "invisible" amongst most other organic functional groups, and the linkage reaction is close to unstoppable, with a combination of strong driving force and singular selective path.



99% for a single step is not sufficient.

Bioconjugation by Copper(I)-Catalyzed Azide-Alkyne [3 + 2] Cycloaddition

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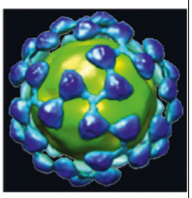
Table 1. [3 + 2] Cycloaddition Reactions of Virus-Azides/Alkynes **1**–**3** with Dye-Alkyne **4** and Dye-Alkyne **5**[e]

entry	reagents[a]	$CuSO_4$ (mM)	**6** (mM)	TCEP (mM)	Cu wire	loading[b]	yield[c]
1	**1** + **4**		2.0	2.0	–	<1 (<2%)	94%
2	**1** + **4**	1.0	2.0		–	<1 (<2%)	80%
3	**1** + **4**	1.0			+	23 (22%)	87%
4	**1** + **4**	1.0	2.0		+	60 (100%)	94%
5	**1** + **4**	1.0	2.0	2.0	–	60 (100%)	96%



0.99^{60} = 55%

96% = 0.9993^{60}

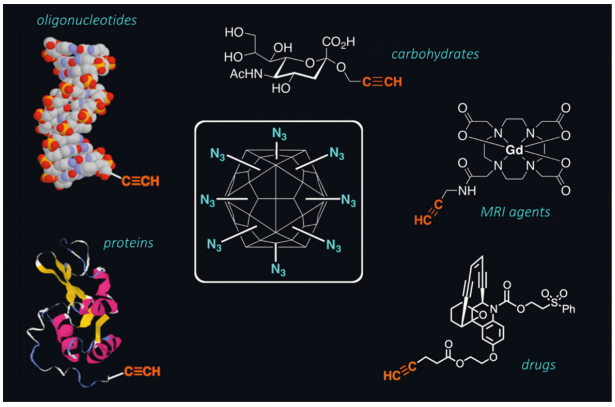


Figure 13. Bioconjugation on a virus using the Copper-catalyzed azide-alkyne cycloaddition.[27]

Such processes are very rare – biology has no need of them – but we do. Qian Wang in M.G.'s lab quickly demonstrated the power of this new reaction by decorating virus particles with dyes, proteins and other agents with complete conversion under very mild conditions (Figure 13).[27] This discovery put us well into unprecedented territory! I could not think of any reaction except olefin polymerizations which could compete, but of course they couldn't be performed in the presence of water or O_2. Publication of this work was delayed so Qian could prove to us in several ways that the outlandish yield implications were correct. To reach the observed 96% yield over 60 linear steps the per-step yield had to be 99.93% [$(0.9993)^{60}$]. Thus, *the border into 'perfect reaction' territory had been crossed*! However, it took the discovery of another such process a decade later before we started talking specifically about perfect reactions.

In 2003, Hartmuth and I summarized the "growing impact of click chemistry on drug discovery" (Figure 14), highlighting that "*the copper-(I)-catalyzed 1,2,3-triazole formation from azides and terminal acetylenes*

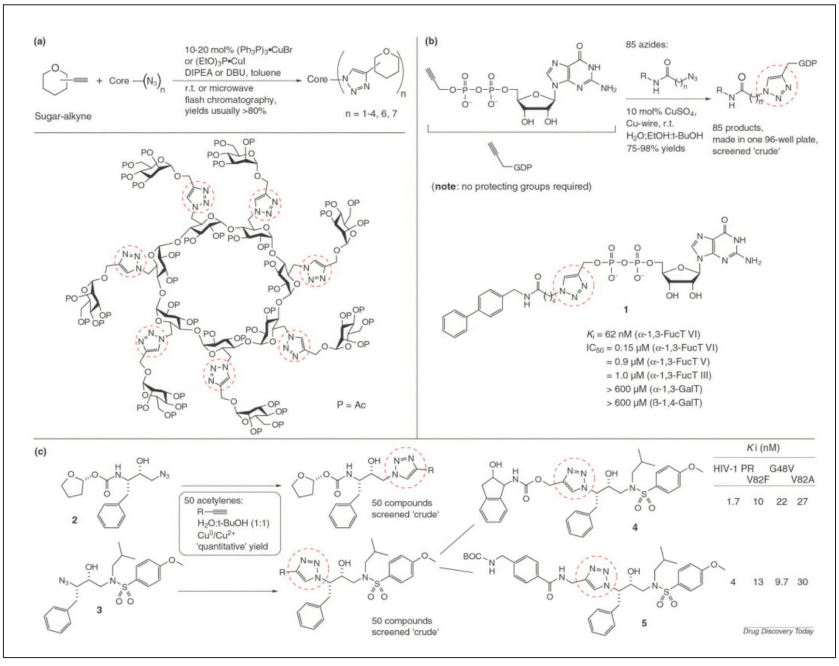


Figure 14. The growing impact of click chemistry on drug discovery (2003).[28]

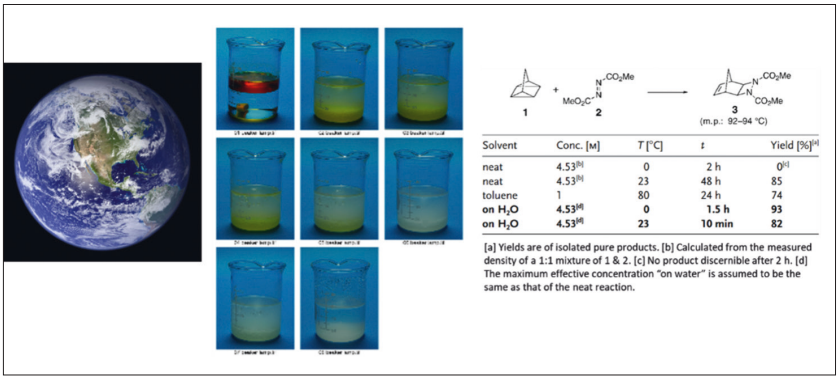


Figure 15. Click chemistry "on" water.[9]

is a particularly powerful linking reaction, due to its high degree of dependability, complete specificity, and the bio-compatibility of the reactants" with "*applications [being] increasingly found in all aspects of drug discovery, ranging from lead finding through combinatorial chemistry and target-templated in situ chemistry, to proteomics and DNA research, using bioconjugation reactions.*"[28]

Later we found that water, life's matrix, is also the best 'solvent' for click chemistry. We observed dramatic rate accelerations for *insoluble reactants "on water", NOT in water* (Figure 15)! Given the giant place for

water on earth and click chemistry's happiness in, on, or under water, this late emergence of interfacial water magic is one of my personally most thrilling, and completely unexpected finds.[9]

The success of click chemistry opened the flood gates for a variety of applications, all enabled by near-perfect *inter*-molecular connectivity.

- ThermoFisher Click-IT™ assays from Salic and Mitchison (2008):[29] "A Chemical Method for Fast and Sensitive Detection of DNA Synthesis *in vivo*"
- Illumina ClickSeq from Routh, *et al.* (2015):[30] "Fragmentation-Free Next-Generation Sequencing via Click Ligation of Adaptors to Stochastically Terminated 3'-Azido cDNAs"
- OLAPLEX using the thiol-ene click reaction,[21] co-founded by UC Santa Barbara materials scientist and click chemist Craig Hawker
- Biomedical imaging by Hartmuth's group, utilizing ligand discovery based on intermolecular linkage builders, *in situ* click chemistry, and rapid radiolabeling by CuAAC (Figure 16).[18,31,32] The team developed the first (and only) FDA-approved imaging agent for Alzheimer's Disease Tau pathology,[18] F-T807 (aka Flortaucipir).[33,34]

Our second near-perfect click reaction, the SuFEx [Sulfur(VI) Fluoride Exchange] process, was discovered by Jiajia Dong (Figure 17).[24] SuFEx is another "magic wand" that forms -SO_2- linkages, the neutral form of Nature's favored -PO_2-- linkage .[24,35] It has chameleon-like properties, very different than the triazole linkage made by azide-alkyne cycloaddition:

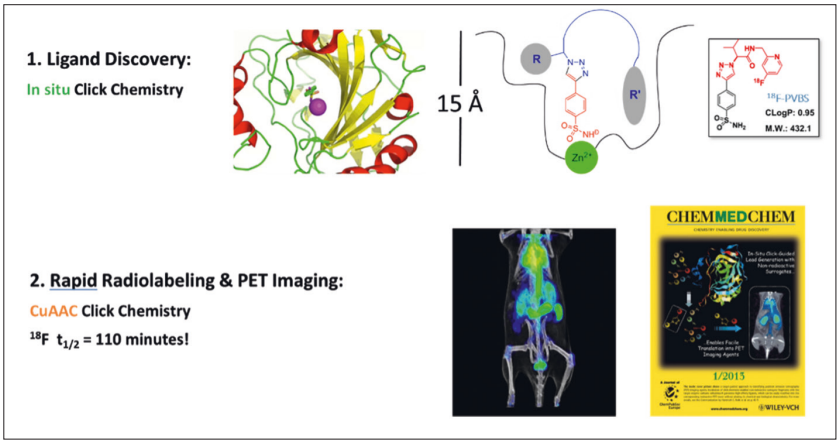


Figure 16. Molecular Imaging enabled by in situ & CuAAC Click Chemistry for Discovery and Radiolabeling. Example: Carbonic Anhydrase Ligands and PET tracer.

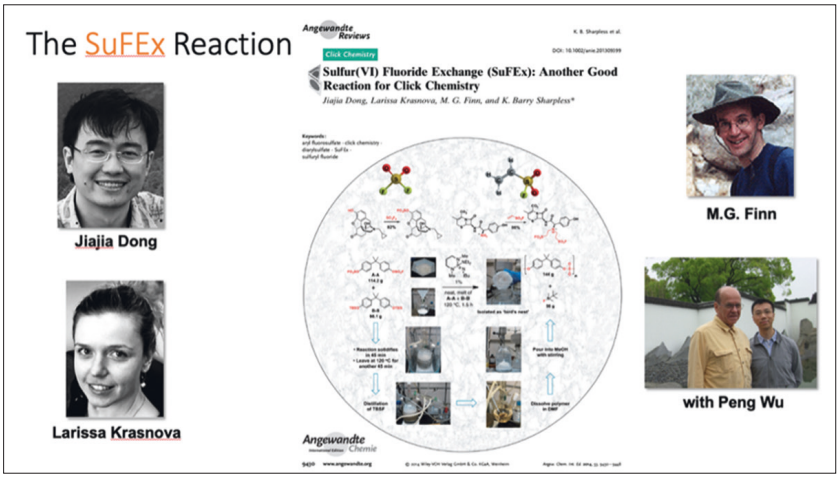


Figure 17. The SuFEx reaction.[24]

stable but controllably reactive, surprisingly nonpolar but able to interact with other molecular fragments, and structurally flexible but thermodynamically strong.

I am incredibly excited about the materials/polymer science opportunities offered by SuFEx because of the last of those characteristics. The making of new polymers (Figure 18) requires hundreds of successive intermolecular connecting steps to occur with extremely high yields in order to give homogeneous, highly pure products. A reaction with 99.999% per step yield will provide 99% yield over 1000 steps, but a small drop to 99.9% per-step yield – which most chemists would consider an excellent reaction – will give an overall yield of just 36.8%! A drop to 99% per step will result in just trace amounts of final product. SuFEx meets this challenge and creates a very interesting type of linkage besides.

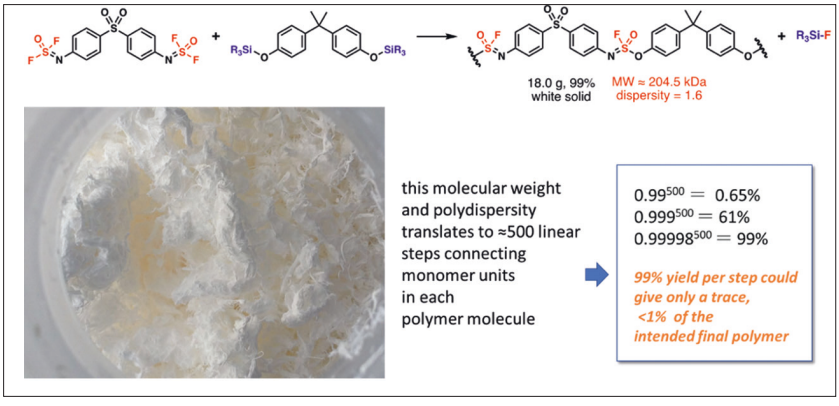


Figure 18. SuFEx – another perfect click chemistry reaction.

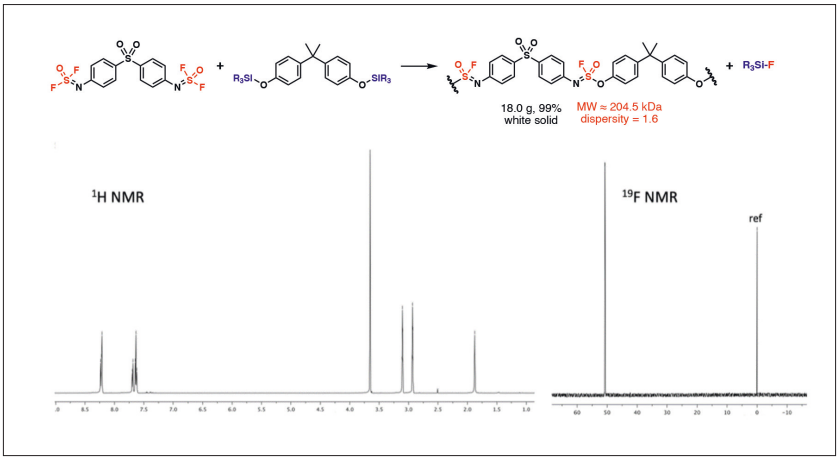


Figure 19. NMR spectra of SuFEx polymer showing only one set of sharp signals. Furthermore, we discovered that (a) the [-N=S(=O)F-O-] polymer backbone linkages are themselves SuFExable and undergo precise SuFEx-based post-modification with phenols or amines to yield branched functional polymers, and (b) that several of these new materials derived from thionyl tetrafluoride had helical polymer structures (Figure 20).[36]

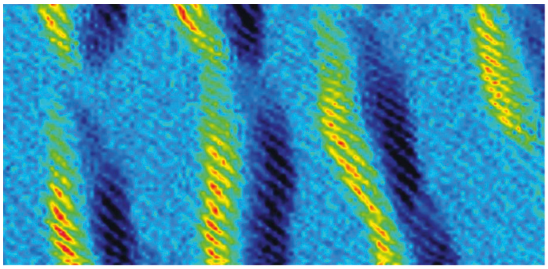


Figure 20. Atomic force microscopy images of thionyl tetrafluoride-based SuFEx polymers, indicating helical coils.[36]

Thus, Suhua Li demonstrated that SuFEx polymerization proceeded with 99.998% yield per step, which resulted in a 500-mer polymer with 99% overall yield. This polymer's ^{1}H and ^{19}F NMR spectra showed only one set of sharp signals (Figure 19), highlighting both the supreme fidelity of each intermolecular connection reaction and the highly dynamic nature of the sulfur-based connectors.

These discoveries have unlocked a whole new world of click chemistry applications in the field of high-temperature, high-field capacitive energy storage (Figure 21). In collaboration with the labs of Peng Wu (Scripps) and Yi Liu (Berkeley Lab), we found a polysulfate polymer that upon coating with ultrathin Al_2O_3 allowed us to produce capacitors that maintained their high electrostatic energy storage performance under thermal and electric extremes (≥ 150°C and more than *700 million volts per meter*!).[37]

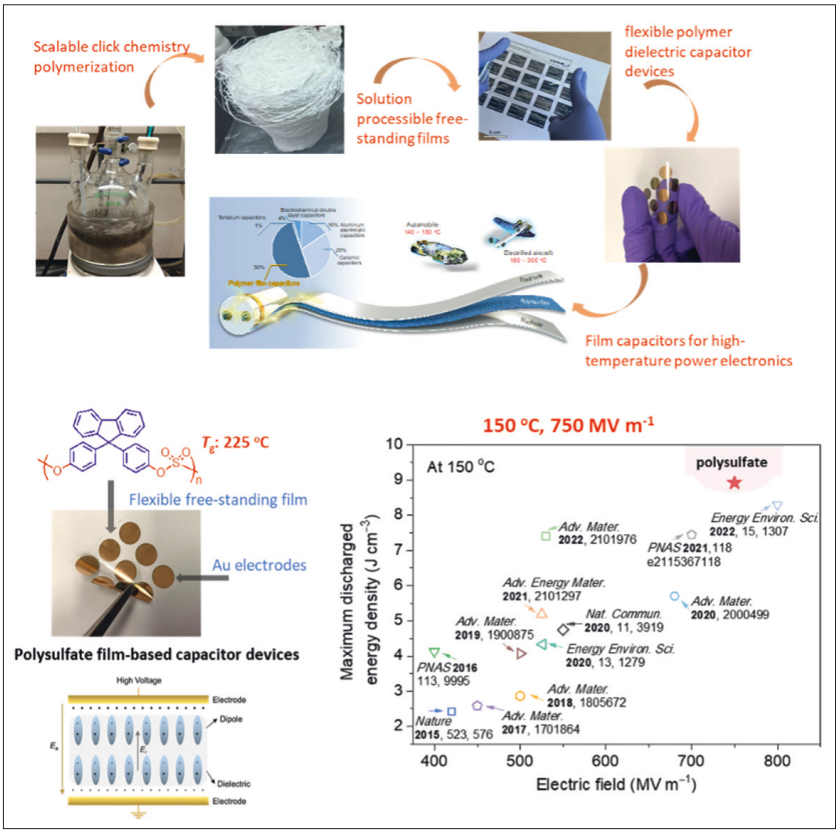


Figure 21. Polysulfates for High-Temperature, High-Field Capacitive Energy Storage.[37]

This journey through the Certainty of Chance, enabled by Click Chemistry, leaves me with several key lessons.

The discovery that *perfect reactions actually exist* (and more are out there waiting to be discovered) is probably the most fundamental certainty-of-chance observation so far enabled by click chemistry.

The faster we can directly create new molecules for screening, the richer will be the flow of new or improved functions.

Properties are more generally modified by control of *inter*-molecular bond connections, not intra-molecular ones. Consequently, fast and reliable inter-molecular connective reactions are the key ingredient in discovery of useful new substances – they enable enormous power in the diversity of modules.

With just a few truly perfect linker reactions we can move extremely fast through structural space and have the best chance to uncover exciting function.

Linkages matter! CuAAC and SuFEx both leave connective functional

groups behind them: triazoles and S(VI) linkages. Beyond their stability under terrestrial conditions, they possess very different properties which are appropriate for diverse applications.

Each new click reaction opens new worlds of discovery by chance.

To our knowledge, there is at present no better method to quickly explore the universe of chemical properties for useful new functional opportunities.

I am extraordinarily grateful to the many coworkers who have joined me on this search for molecular function and chemical reactivity.

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