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From Bioorganic Models to Cells

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Abstract

I endeavor to share how various choices—some deliberate, some unconscious—and the unmistakable influence of many others shaped my scientific pursuits. I am fascinated by how two long-term, major streams of my research, DNA replication and purine biosynthesis, have merged with unexpected interconnections. If I have imparted to many of the talented individuals who have passed through my lab a degree of my passion for uncloaking the mysteries hidden in scientific research and an understanding of the honesty and rigor it demands and its impact on the world community, then my mentorship has been successful.

Contents

THE BEGINNING

I was born on April 20, 1938, in Orange, New Jersey, a date I share with a number of notables (Joan Miro, John Paul Stevens, Lionel Hampton), but unfortunately, all of us are overshadowed by Adolf Hitler. According to the hospital record and snatches of conversation with my mother, my entry into the world was especially painful for her, since I was a breech birth not born by a caesarian section. My parents were married during the Great Depression that disrupted my father's plans to practice law, and his service in World War II ended that dream. But, he was the first in his small community (Danielsville, Pennsylvania) to attend college (my grandfather had made his money as a bootlegger), and as a result, I had access to a chest of books, one of which was a college chemistry book.

My life as a scientist really began when we moved to Stamford, Connecticut, where our family (I now had a brother) owned our first house with some empty lots surrounding it. Although no one in the family genealogy was identified as a scientist, I became one. By the time I was in junior high school, I loved identifying flowers, trees, clouds, rocks, and birds—my allowance was spent on various guidebooks—and during summers spent on my grandmother's farm, I collected and cataloged all kinds of objects (wild flowers, minerals, leaves, fossils, etc.) and built my first scientific instrument, a hygrometer (out of my grandmother's long hair) to measure the humidity on those sultry days in central Pennsylvania. By high school, I began my chemical experiments. Reluctantly, my parents had gifted me a Gilbert chemistry set for Christmas, but as others in their memoirs have noted, the range of chemicals and suggested experiments were too confining. I knew, however, what I needed from that college chemistry text, and so I made the rounds of drug stores, wrote for free samples, and acquired the reagents I needed.

Discarded car batteries were an especially good source of sulfuric acid and elemental zinc. I was producing hydrogen and had rigged a bicycle pump so I could inflate balloons with a few liters of the gas. In those falls, one could still burn the raked leaves whose smoldering embers ignited my primitive dirigibles. The open lots (Stamford was not yet paved over) provided an area for rocket launches and testing explosive mixtures created from calcium carbide and water to produce acetylene; the cellar tool bench was a platform for compound synthesis and mineral analysis. I was on my way.

I attended Saint Basil's Preparatory School in Stamford, Connecticut, which was focused on preparing young men for ordination in the Byzantine rite of the Catholic church. The curricula were heavily concentrated on languages and literature, with little science, and only the necessary, mathematics. But by this time, I had learned enough chemistry to pass the Junior final chemistry exam within the first week and was granted some freedom to do my own experiments. I promptly improved my rocket propellant. Nevertheless, a love of literature and my acquisition of a strong foundation in writing skills was Saint Basil's legacy.

LEHIGH DAYS

Much to my father's chagrin (as a Lafayette alumnus), I chose Lehigh over Lafayette because I had decided on becoming a chemical engineer. In the freshman placement exams, I did well enough in mathematics to qualify for the chemical engineering program, but in the writing of an original essay, I excelled. I was invited to be an English literature major as well as an engineer, and the Dean's Office mapped out a five-year course to achieve a double degree. By the second year, I had been captivated by chemistry laboratory courses that used reagents I had only wished to have on my cellar bench. I switched to major in chemistry and English literature. I had the good fortune of establishing close relationships with two professors, Robert Young, a biochemist, and Jerome Daen, a physical chemist. With the latter, I learned the importance of controls, exacting precise measurements, statistical significance, and reproducibility. With the former, I studied the myriad metabolic pathways, the structures of the associated metabolites, and the mysterious proteins (enzymes) that were responsible for the metabolic transformations detailed in the venerable textbook by Fruton & Simmonds (1). The first enzyme (jack bean urease) had been purified three decades earlier (2), but investigations as to how enzymes catalyzed their respective transformations were just stirring (for an example, see 3). In the Young laboratory course that focused on organic compound identification, I delighted in the opportunity to synthesize derivatives with a variety of reagents that, unfortunately, for safety and/or environmental reasons, are no longer found in undergraduate labs. It was my introduction to wet chemistry and its valuable laboratory techniques.

In the late 1950s, Lehigh was a monastic order prone to boisterous campus partying on weekends. There were no dating services, so coeds were often met through blind dates. On one such occasion, I met a vivacious young woman, Patricia Doran, a senior majoring in chemistry at Chestnut Hill College in Philadelphia. Our courtship was enabled by repeatedly borrowing my fraternity brothers' cars and heavy use of the telephone. The nuns held the young women to strict curfews, so dates often ended in a race to return to campus before the driveway gate closed. After a year at DuPont, Pat would join me at Cornell, where I planned to begin graduate work.

OFF TO CORNELL

Jerome Daen had encouraged me to apply to Cornell for graduate school because of its faculty strengths in physical chemistry. Cornell offered me a Teeple Fellowship that meant I would start graduate research almost immediately. I accepted. I met with many prospective faculty mentors, the last one being a new arrival, Tom Bruice. My first impression was of a man of excess energy, intensely focused, driven, and competitive. He proposed understanding the mechanism of enzyme action through the construction of ingenious organic compounds that juxtaposed functional groups and substrate fragments to mimic an enzyme's active site. The idea was simple: Would, for example, a correctly positioned imidazole adjacent to an ester group within the same molecule catalyze the hydrolysis of the ester? If so, what was the mechanistic pathway? Note that

at the time, popular organic texts still viewed ester and amide hydrolysis as limited to the purview of hydronium and hydroxide ion chemistry.

Because of the fellowship, I started research in the spring of my first year. We had decided to synthesize a series of molecules that featured an amine molecule on one end and a reactive aromatic ester on the other end that upon closure gave five- or six-membered lactam rings. It was pure physical organic chemistry with a biochemical twist—quintessential bioorganic chemistry. The desired compounds cyclized so rapidly when in aqueous solution that I couldn't follow the liberation of the phenolic product in a conventional Zeiss spectrophotometer. At the time (1962), there was some buzz about a stopped-flow instrument, but none were commercially available. So, I had to build my own, but a much more important event soon filled my consciousness—Pat and I were to marry at the end of the semester. Tom was clearly not happy about the interruption, especially one that involved a honeymoon at Virginia Beach, miles from Ithaca.

After the short stay in Virginia Beach (Pat had suffered a severe case of sunburn), our two fathers provided me with access to what I needed for a stopped-flow instrument: a fine machinist in Stamford and a block of special stainless steel from Bethlehem Steel. So, we prolonged our honeymoon, and I had built a stopped-flow instrument to fit into a Zeiss spectrophotometer before returning to Ithaca. I received a very icy reception from Tom, until I unpacked my treasure. Within a few hours, most of the Cornell faculty had paraded past my bench and viewed a demonstration by Tom of how the hydrolysis of my esters could be tracked on a millisecond time scale. How primitive the device is in retrospect (4)! But its operation led to my first *Journal of the American Chemical Society* paper (5), which anchored my thesis.

The three year stay at Cornell passed quickly. The bioorganic stars of that era all visited (Myron Bender, Frank Westheimer, Bill Jencks), and their competitive natures were palpable. Long lab hours were expected. Tom had a rifle-like arm for passing a football, and tensions were relieved on some afternoons with touch football, often leading to dislocated fingers. My thesis work proceeded rapidly; Pat, who had been employed by Tom as a technician, conducted many of my needed kinetic experiments, and her extraordinary laboratory skills first impacted my graduate career and later my own laboratory—more to be said about that in the Section titled The Transition from Bioorganic Chemistry to Enzymology (1965–1970). In truth, she merited a PhD as much as me. By now Tom, being a native Californian, was growing weary of the Ithaca winters, so on one snowy day, he brought all of us together and told us to pack, for we would be setting up his new lab in Santa Barbara. Westward Ho!

CALIFORNIA INTERLUDE

I had finished my PhD work at Cornell and made a momentous decision. As Tom had said, "This will either be the start or the end of your career," so my postdoctoral project was to remain with Tom and author our two-volume text, *Bioorganic Mechanisms* (6, 7). I had started on our proposed opus while at Cornell, digging out pertinent papers from the journal stacks in the Baker and Olin Libraries. Recall this was the time of no digital indices and no Google but yellow pads and numerous index cards. I inhaled much dust, but as the number of cards grew, the text of the chapters started to take shape.

Tom had already sketched an outline of the text in his graduate course. We intended to cover all of the published work that one could construe as bioorganic chemistry. I was also beginning to jot down ideas for research. Although I had interviewed both in industry and in academia, I had not yet decided on what career course to follow. During an interview at DuPont Central Research, I ended the day with the Director, Howard Simmons. He said, and I quote, "We'd love to have you, but you belong in academia." His statement certainly influenced my decision. Although I had an offer of an assistant professorship from Penn State, I still hesitated and decided to put

off a decision until the writing was finished. I spoke with the head of the chemistry department, Thomas Wartik, who wisely recognized my need for more time and made the offer open-ended (no deadline). Tom, who became not only a colleague but a very close friend, patiently on occasion called me in Santa Barbara and asked how the writing was proceeding. In the interim, my curiosity to test out some of my growing list of research ideas catalyzed my acceptance of the Penn State offer. Santa Barbara is a jewel nestled next to the Pacific, and now Pat and I were leaving for the wilderness of rural central Pennsylvania to set up a lab. Many of our lab mates questioned our choice. I had one major task to finish, indexing the two-volume text of *Bioorganic Mechanisms*. We were Eastward bound.

THE TRANSITION FROM BIOORGANIC CHEMISTRY TO ENZYMOLOGY (1965–1970)

My first lab at Penn State was in a space that had housed an analytical chemistry course, so the desks all held wonderful balances but nothing else. I should have been wise enough to retain several, for they are now collector's items. On one end was a small hood and, in the center, a glass-enclosed office with a desk under a rain gutter because of a leaking pipe. My "million dollars" of startup was a pH meter and access to stockroom glassware. But before I left Tom's lab, I had written a National Institutes of Health (NIH) grant application based on the idea that many biological substrates, especially those involved in phosphoryl transfer, had built-in catalytic groups, an example being the carboxyl group of phosphoenolpyruvate. To my surprise and delight, it was funded! My first graduate student, Keith Schray, was extraordinary (he went on to head the Department of Chemistry at Lehigh) and before long he had data implicating a pentacovalent phosphorus intermediate in the transfer of the phosphoryl group from phosphoenolpyruvate to other receptors. Our papers (8, 9) caught the attention of Frank Westheimer, who followed our research from then on. I started a second project based on creating models for the tetrahydrofolate cofactor whose ability to orchestrate one-carbon transfer chemistry at various oxidation levels when bound to cognate enzymes had intrigued me (10, 11). A third area was that of sulfate esters (12, 13): Did their hydrolysis proceed through sulfur trioxide, a stable species contrasted to the fleeting metaphosphate whose presence in phosphate ester hydrolysis was hotly debated at Gordon Conferences and European meetings? Those latter papers had a coauthor, Pat, whose experimental and managerial skills undoubtedly laid the groundwork for all of the lab's future success and reputation. She implemented laboratory practices that are now a tradition and adopted by departing members for their own labs.

But model compounds are not enzymes, so I decided, with no background in protein chemistry, to plunge into isolating fructose 1,6-bisphosphatase from rabbit liver. With Margaret deMaine, an inorganic chemist with a doctoral degree from Cambridge, and Jake Kleinschuster, a returning Vietnam veteran, we learned how to grind up liver, carry out ammonium sulfate fractionation, pour our own phosphocellulose columns, and isolate purified protein (at least by sodium dodecyl sulfate gels) (14, 15). We were rank amateurs, not knowing initially to remove proteases by a heat step. I chose this enzyme as an example of one that catalyzed phosphate ester hydrolysis in an important metabolic pathway, glycolysis. But I realized soon that, whereas one knew the chemical structure of the substrate, I had no idea as to the active isomeric species; was it the α- or β-anomer or the open-chain keto form? All of the textbooks showed the sugars in glycolysis to be in the α-anomeric form, but that, I discovered, was assumed not proven. I decided I needed more immersion in enzymology, so Pat and I took up residence for 3 months at the Cancer Institute in Philadelphia, where we rejoined Keith Schray and shuttled between two labs, those of Irwin "Ernie" Rose and Al Mildvan.

ANOMERIC SPECIFICITY (1972–1976)

Those of you who knew those two scientists would suspect it was enzymology day and night, and you would be right. Ernie would call me at all hours to discuss his latest ideas on proton exchange, a new scheme to detect the presence of transient intermediates by pulse-chase methods, and I could go on. Ernie's ideas were all intuitive, and I often puzzled over where they had originated. In the corner of his lab were parts that would assemble into a primitive rapid-quench machine, a gift from Al Meister with no instructions that also came with a number of his cigar butts. I figured out how to assemble it. The machine used a cam shaft that spun at different speeds, and the cam would strike a set of syringes, driving their contents into a cell. A delay of different lengths varied the time of quench. We broke a number of syringes but ultimately produced a series of biphasic plots that revealed which isomeric form of the sugar was being consumed by excess enzyme. In the meantime, Al Mildvan, working with Keith Schray, was able to determine the distribution and rates of anomeric interconversion for various sugars by nuclear magnetic resonance (NMR). In the case of fructose 1,6-bisphosphatase, both matched our quench studies on this sugar implicating the α-anomers as the enzyme's substrate. We then went on to elucidate the anomeric specificity of all of the enzymes in the glycolytic pathway (16–20).

TRANSITION FROM MODELS INTO THE TRANSFORMYLASE AND HYDROXYLASE ENZYMES (1976–1984)

Our studies on the mechanism of action of folate- and biopterin-requiring enzymes focused on the transformylase enzymes in de novo purine biosynthesis (21–26) and phenylalanine hydroxylase (27–32). All were purified the old-fashioned way by extraction from minced livers and fractionation with ammonium sulfate followed by cationic or anionic column chromatography. We noted that the transformylase enzymes in the de novo purine pathway appeared to purify together (22), a finding that was not convincingly confirmed until decades later. We found that the cofactor for both transformylase enzymes is 10-formyltetrahydrofolate (23, 33), that the one-carbon unit was directly transferred (34), and that the human glycinamide transformylase is a trifunctional enzyme (35). For phenylalanine hydroxylase, we demonstrated by ^{13}C NMR that the tetrahydrobiopterin is converted during the hydroxylation to a 4a-carbinolamine, thus unequivocally determining the atom of attachment of molecular oxygen to the tetrahydrobiopterin (28), that the phenylalanine hydroxylase stimulator protein is a 4a-carbinolamine dehydratase (36), and that the enzyme from *Chromobacterium violaceum* is a copper-containing hydroxylase (37).

To transition from a coarse grain to a sharper view of an enzyme mechanism required our creative adaptation of a variety of probes for a wide range of methods: stopped-flow and quench-flow kinetics to determine the overall kinetic sequence, isotope effects to pinpoint the locus of the rate-limiting step, and measurements from the stereochemical course of the transformation to infer the presence of transient intermediates (28, 38–44). But what enzymes should be subject to such scrutiny? The reactions they catalyzed should be of great biochemical significance, the proteins stable and easily accessible in quantity and amenable to the aforementioned protocols. A fortuitous short sabbatical helped me in my choices.

DIHYDROFOLATE REDUCTASE AND DNA POLYMERASE (1980–)

I had the immense good fortune to spend 6 short months in the legendary biochemistry department headed by Arthur Kornberg at Stanford University, hosted by George Stark. It was a heady, exciting time. George was perfecting Northern blots. Paul Berg had demonstrated the feasibility

of recombinant expression of human genes in bacteria, and the intense departmental seminars revealed weekly discoveries and novel techniques. And then there was the opportunity to lunch on toasted cheese sandwiches with Arthur and hear his thoughts on "his" polymerase enzymes.

I returned to Penn State with fresh enthusiasm, having made key decisions. The lab would have two foci: the mechanisms of DNA replication and folate-requiring enzymes. We began with the Klenow fragment of DNA polymerase (45–51), first delineating its minimal kinetic scheme for replication using rapid-quench methods, revealing its high proofreading activity from the perspective of its free energy profile by identifying two key rate-limiting conformational changes flanking a rapid internal equilibrium, and mapping the distance between its substrate sites by fluorescence resonance energy transfer and time-resolved fluorescence spectroscopy. The existence of a non-rate-limiting chemical step in enzyme-catalyzed reactions is now accepted as a general characteristic. But the Klenow fragment polymerase from *Escherichia coli* is not responsible for DNA replication. Now that we were more sure of our abilities to prepare and handle enzymes and of the power of our arsenal of methods to reveal the intimate details of how an enzyme functions with complex substrates and activities, we began our studies of the T4 replisome. Inspired by the fundamental discoveries of Alberts (52, 53) and Nossal (54) in isolating and characterizing the roles of the eight proteins that constitute the T4 replisome, we started by studying the functions of the individual proteins, their various subcomplexes, and finally the intact replisome—all in vitro. I was particularly fascinated by how all of their functions were ultimately integrated into a functional replisome. This research adventure became an odyssey that spanned nearly three decades (55–92). It was an exciting experience for us as we progressed to applying new techniques such as single-molecule total internal reflection fluorescence and molecular tweezers to answer questions that had been heretofore inaccessible. With Michelle Spiering, who was instrumental in the success of many T4 studies, I recently wrote a *Journal of Biological Chemistry* review article (92) that summarized all of our studies. What remains is the actual structure of the replisome, which I hope will be attainable through cryo–electron microscopy. Although T4 is from phage, many of our findings have applied to eukaryotic replisomes, and our work served as an entry into many stimulating conferences—Cold Spring Harbor, Gordon, UCLA, etc.—where many lasting friendships and collaborations were formed, often in rustic settings.

An invitation to speak at The Gordon Conference on Enzymes, Coenzymes, and Metabolic Pathways was always highly coveted. Every early July, the world's leading enzymologists gathered there, sat in the front row of a 200-seat auditorium, and engaged in heated debates about the merits of the various talks. For young assistant professors, to speak there was a rite of passage—one now-famous biochemist stepped back too far and disappeared off the stage, another hyperventilated—but in the evening, all gathered in the cafeteria for a night of libations. Pranks abounded, beds were short-sheeted, talk slides were mysteriously shuffled and at times replaced with photos found in *Playboy*, and fireworks were ignited in the bar. I went to that meeting for many years until the beds and mosquitos became too uncomfortable.

In parallel with our studies on the T4 replisome, the other half of the lab had embarked on defining the number and magnitude of the kinetic steps that characterized the turnover of dihydrofolate reductase. I had chosen this enzyme for the determination of the free energy profile of the catalytic cycle. High resolution crystal structures were available from the exacting studies of Kraut and his colleagues (93), the enzyme was small and amenable to NMR investigations, and the reaction involved a hydride transfer that could be probed by H/D isotope effects. I felt the solution lay in the application of stopped-flow kinetics. At that time, only the free energy profile of triosephosphate isomerase had been revealed by the elegant experiments of Rose (94) and Knowles (95); in contrast, dihydrofolate reductase was a bisubstrate enzyme that provided novel challenges. Thanks to Carol Fierke's exacting experimental skills and analytical reasoning abilities, the free

profile for the turnover cycle of the dihydrofolate reductase was solved (96). That solution, along with the X-ray crystal structure, served as the basis for a series of extensive mutagenesis studies using ensemble and single-molecule kinetic approaches to identify key residues within and distal to the active site that participated in the catalytic cycle (97–107). It soon became apparent that the entire protein structure contributed to varying extents to the catalytic efficiency of the enzyme. A striking feature of the dihydrofolate reductase kinetic scheme recalled that of the Klenow polymerase, namely a free energy profile featuring rate-limiting steps associated with conformational changes within the protein, surrounding a fast chemical step. In short, the step involving chemistry carried out by the enzyme had been optimized by evolution. I found this general concept deserving of further scrutiny and validation.

CATALYTIC ANTIBODIES AND SCRIPPS (1988–)

In the mid-1980s, I chaired the Penn State Summer Molecular Biology Symposium and invited Richard Lerner to present a lecture. Lerner (108) and Peter Schultz (109) had just reported their discovery of catalytic antibodies, confirming Pauling's hypothesis that enzymes gained much of their catalytic prowess by stabilizing the transition state for the reaction in question. In the Lerner study, catalytic antibodies had been obtained by generating monoclonal antibodies against a hapten constructed to resemble a transition state for the chosen reaction. The key questions asked were, What classes of reactions would be catalyzed by antibodies, and how closely would their turnover approach that of enzymes? Richard invited me to the Scripps Research Institute for a 3-month sabbatical to address these issues, which marked the first of many stimulating and invigorating mini-sabbaticals I've enjoyed there (Pat and I also escaped central Pennsylvania's winters). Over only a few years, Richard had transformed Scripps to take a leading role in organic chemistry and had hired a number of luminaries (Nicolaou, Sharpless, Rebek, and Bolger, as well as promising young chemists including Hilvert, Cravat, and Kelly). After-hours gatherings at the Faculty Club were intense and stimulating.

At Scripps we generated catalytic antibodies for facilitating a large variety of reactions: ester and amide hydrolysis, cyclization, peptide synthesis, and decarboxylation, among others (110–127). Methods were developed to assemble combinatorial antibodies on phage surfaces in a quest to find antibody candidates with superior activity (120, 128, 129). One antibody that catalyzed amide hydrolysis was found to proceed through an acyl antibody intermediate, like its enzymatic counterpart (122, 123, 130). Despite our best efforts, which included extensive mutagenesis, the achieved rate stimulations were similar to those found upon comparison of paired intramolecular and bimolecular reactions—rates several orders of magnitude less than those for enzymes. As more structures of antibody-hapten complexes became available, it was obvious that the penalty for the tight hapten-antibody binding in the rigid antibody structure was manifest in the inability of the structure to adapt to the chemical changes in the substrate over the course of the reaction. Dissociation of the reaction products that would require a conformational change in the antibody structure was often slowed.

Our earlier studies on dihydrofolate reductase had implicated conformational changes as key to the enzyme's catalytic efficiency. It was possible to observe directly the regions of the enzyme showing conformational movements and their rates through NMR relaxation measurements. Being at Scripps provided me the opportunity to discuss this application with Pete Wright and Jane Dyson, which led to a long-standing collaboration and close, personal friendships. NMR assignments to the amino acids in dihydrofolate reductase (131, 132) followed by relaxation measurements (133) later culminated in a demonstration that the steps constituting the catalytic cycle determined by stopped-flow kinetics could be assigned to conformational fluctuations in specific

regions and residues within the protein. These regions and residues also responded to site-specific mutations (134, 135). Importantly, the rates of these movements, as measured by NMR, beautifully matched those of the stopped-flow kinetics.

Despite the rapid development of diverse experimental tools to probe enzymatic catalysis more finely, it became apparent that theory would unlock even more secrets (104, 136, 137). We applied in-depth dynamic simulations to dihydrofolate reductase through an extraordinary collaboration with Sharon Hammes-Schiffer (138, 139). It was a wonderful learning experience for me and, in my view, definitively showed the relationship between an enzyme's conformational changes and the catalytic cycle. The simulations showed that dihydrofolate reductase, through a series of step changes within the protein structure both distal to and within the active site, constructed an interactive space complementary to the transition state, so the actual chemical transformation step would not limit the reaction cycle. We were joined by Gordon Hammes, long a close friend, and with them, I published several of my most highly cited papers (140–146).

METABOLONS, THE PURINOSOME (2008–)

The lab continued to delve into other folate-requiring enzymes, in particular, the transformylases involved in eukaryotic de novo purine biosynthesis. We had found that the human glycinamide ribonucleotide transformylase was one activity of a trifunctional protein (35). With my Scripps connection, we worked with the Bolger and Wilson labs to obtain crystal structures of the more readily obtained *E.coli* enzyme docked with various substrate analogs (147–154). I was still tantalized by our decade-old observation that the eukaryotic transformylases from avian liver copurified. We had unsuccessfully tried a number of in vitro studies searching for evidence of a complex between the two transformylases and other enzymes in the de novo purine biosynthetic pathway. Now, with the availability of the eukaryotic genes and the ability to create chimeric fluorescent proteins, we searched in HeLa cells for a minimal complex between a transformylase and one of the other five proteins in the de novo pathway. The result was our discovery by Songon An (155) of the purinosome in HeLa cells. Subsequent work uncovered an endogenous assembly of the six enzymes that convert the sugar ribose-5-phosphate-1-pyrophosphate to inosine monophosphate through a channeled process (155–161). This complex has all of the characteristics of a liquid condensate and is the first example of a dynamic metabolon. I continue to research its properties.

A PARALLEL COURSE (1970–)

With the advent of bioorganic chemistry and its implications for rational drug discovery, major pharmaceutical companies soon reached out to our community. I was contacted by SmithKline & French (SKF, now GlaxoSmithKline) and asked to consult for their medicinal chemistry group. Their drug discovery effort was heavily focused on screening their collections of natural products against various models of disease. As part of my responsibilities, I presented a short course on mechanistic enzymology (captopril's discovery as an angiotensin-converting enzyme inhibitor had just jolted the traditional drug discovery approach). With the arrivals of George Poste and Stanley Crooke, medicinal chemistry research underwent profound changes. Since I reported to George, a polymath by every measure and soon to be in charge of worldwide SKF research, I became involved in all aspects of the company's research efforts. Soon Beecham was merged into SKF to form SKB, and my consulting expanded to include numerous trips to the United Kingdom. I had an unusual opportunity to be involved in the reshaping of SKB's ongoing basic research, in the assessment of clinical trial results, and ultimately, in strategic planning. Not only did I report to George but now also to Jan Leshley, the CEO at the time. It was for me an opportunity to

step into another universe where excellent science had to be weighed for its commercial value; large egos and clashing personalities had to be cajoled or commanded into agreement; and failed, risky, decisions had repercussions on Wall Street. Rather than the usual show-up-once-a-quarter consultantship, I was on call and possessed a secure computer detailing all SKB's research projects and clinical trials. This experience would later prove invaluable.

As SKB grew, George decided to create and expand various Advisory Boards. I now headed the Scientific Advisory Board (SAB), a position I held for 25 years. I brought on many outstanding academics who introduced SKB to new developing areas ripe for drug discoveries. Two have remained lifelong, special friends: Sid Hecht, already a vice president at SKB, and Lucy Shapiro, about whom I have much to say later. It was a heady time for all who participated; George is a nonpareil raconteur (his imitation of an English lord is a showstopper), and the resulting scientific camaraderie always left me inspired for discovery research. With the final merger to GlaxoSmithKline, the resulting behemoth was almost unmanageable, and it was time to move on.

I now became involved in consulting for two key companies in the nonpharmaceutical world, DuPont and Corning. Joseph Miller, who was a notable graduate student in the first course I ever taught (Joe told me after my second lecture to slow the presentation), was successively Chief Scientific Officer at DuPont and Corning. At DuPont, their programs focused on modifying enzymes to produce high-value industrial, commercial products; for Corning, their goal was to develop biological applications for their unique materials. In hindsight, Joe's vision was too early, and such companies, with their high-capital investments, were not ready to abandon their traditional synthetic routes or risk unexpected liabilities from new products.

During my tenure at SKB, one of my joys was discussions with other SAB members. Over breakfast at the Four Seasons Hotel, Lucy Shapiro and I discussed the inevitable development of antibiotic resistance. She was immersed in research on her model organism, *Caulobacter crescentus*, and had discovered a key DNA methylation enzyme required for its life-cycle progression. If this enzyme could be inhibited, perhaps there would be a reduced probability of resistance occurring. One of my postdocs, Steve Baker, took up the challenge and created a small combinatorial library of molecules whose structures were built around boron. Several were inhibitors not only of the DNA methyltransferase but also were cytostatic against pathogenic bacteria such as *Bacillus anthracis* and *Brucella*.

Lucy and I jointly presented our findings at a conference held at Fort Detrick in Maryland. In the audience were representatives from the Defense Advanced Research Projects Agency, who within a day approached us to submit a proposal in the national interest. Within the astonishing short time of 2 weeks, I was called and told that our collaborative effort would be funded, provided we agreed to form a biotech startup. Most fortunately, our collective consulting experiences had provided us with a network of contacts, so with some trepidation we agreed, and Anacor was formed. Quickly, lab space was rented in Palo Alto; the CEO, David Perry, and other personnel were hired; and additional venture-capital funds were brought in. Anacor became my parallel career for the next decade.

To differentiate Anacor from others, we chose to build a combinatorial library centered on boron, against the advice of many experts (boron was too toxic), and to eschew preselected targets for phenotypic screening. Anacor quickly learned that the majority of its library compounds were devoid of toxicity in cellular and animal models and also possessed potent antifungal activities. So, the Anacor journey began, guided by the intense focus of David Perry, who deserves much credit for its success. The road traveled was curved and full of potholes, such as the failure of a lead candidate, owing to resistance; contentious board meetings over strategy; and the continued, nagging need for money. But after a decade, Anacor had registered two drugs, and now with Paul Berns as CEO, Pfizer swooped in and bought the company. Among cherished mementos are letters of

gratitude describing how crisaborole, Anacor's atopic dermatitis drug, cleared the red rash, relieved the itching of eczema, and stilled the crying of infants. Postdoctoral fellows who were named on the original patents, and among whom I had divided the share pool, were amply rewarded.

After the sale of Anacor, Lucy and I felt the boron platform had much more to contribute to human health and well-being. I, with a superb postdoc, Tony Liu, set out to explore whether a new library based on boron would exhibit antifungal properties against fungal pathogens that devastate key world food crops such as soybeans, corn, and rice. Aided by Paul Schimmel, long a close friend, excellent scientist, and biotech entrepreneur extraordinaire, we established Boragen. At the time of writing, Boragen's field trials of an unusually effective antifungal agent are being run in various areas of the world where resistant fungal diseases can drastically lower the yields of the aforementioned crops. I hope for another success.

REFLECTIONS

As I look back over my long career, it might prove instructive to others to view the path taken, particularly its continuing evolution from simpler to more complex systems. My work reflected my captivation by bioorganic models (**Figure 1**) initially and by more complex cellular biology (**Figures 2** and **3**) more recently. The laboratory skills required also underwent a metamorphosis. I always have been motivated by an abiding passion to deepen our understanding of how biological systems function. So, as new methods were invented that could unlock novel insights, my lab incorporated them.

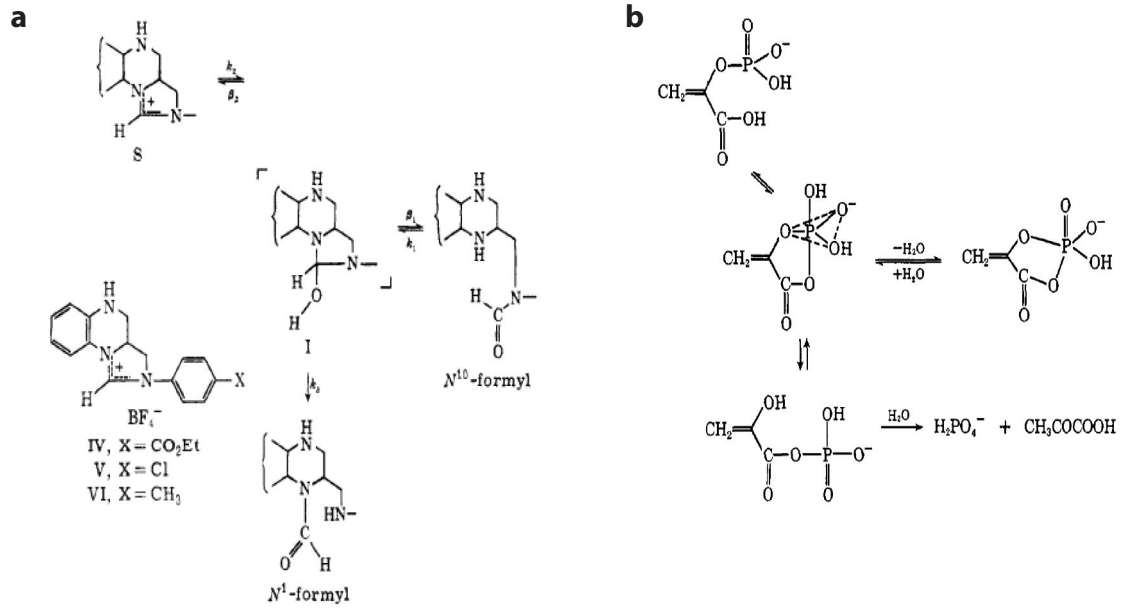


Figure 1

Bioorganic models (early career). (*a*) The hydrolysis of the methenyl salts of various tetrahydro quinoxaline derivatives as a model for the hydrolytic behavior of the *N*5,*N*10-methenyltetrahydrofolate cofactor. (*b*) The hydrolysis of phosphoenolpyruvate, showing how the internal carboxylate activates the phosphoryl group for transfer. Panel *a* reproduced from Reference 11. Panel *b* reproduced from Reference 9.

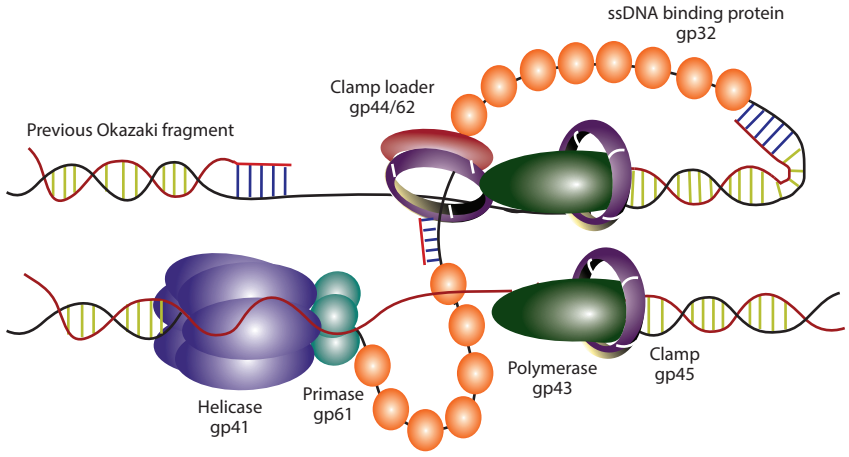
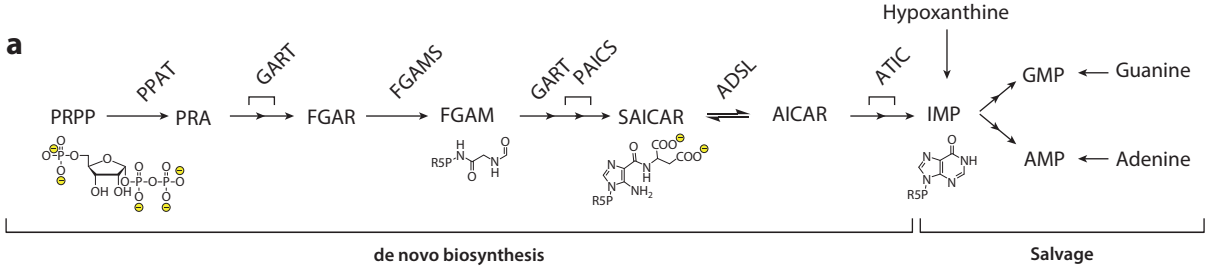


Figure 2

T4 replisome (late career). A representation showing seven of the eight proteins, minus the helicase loader gp53, that constitute the T4 replisome. Abbreviation: ssDNA, single-stranded DNA.



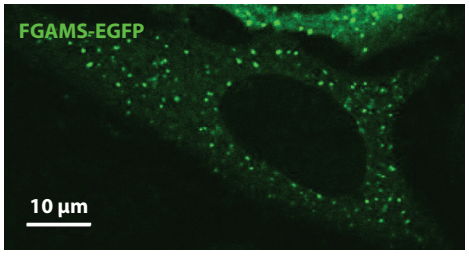


Figure 3

De novo purine biosynthesis (late career). (*a*)The multienzyme pathway for de novo purine biosynthesis. (*b*) A fluorescent image of purinosomes in HeLa cells. Abbreviations: ADSL, adenylsuccinate lyase; AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranosyl 5′-monophosphate; ATIC, 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase; EGFP, enhanced green fluorescent protein; FGAM, formylglycinamide ribonucleotide; FGAMS, formylglycinamidine ribonucleotide synthase; FGAR, formylglycinamide ribonucleotide; GART, glycinamide ribonucleotide transferase; IMP, inosine monophosphate; PAICS, phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase; PPAT, phosphoribosyl amidotransferase; PRA, 5-phosphoribosylamine; PRPP, phosphoribosyl diphosphate; SAICAR, phosphoribosyl aminoimidazole succinocarboxamide. Figure adapted with permission from Reference 162.

I was fortunate to enjoy enlightened leadership at Penn State, notably from Tom Wartik, first as chair of the Department of Chemistry and then dean, Joe Dixon as chair of the Department of Chemistry, and Bill Richardson as provost. As the first member of the department with a biological bent, I was fairly isolated; only later did Gordon Hamilton, Joe Villafranca, and Bob Matthews arrive to help build a national bioorganic presence. I particularly benefited from a collaboration involving surface ionization mass spectrometry with Nick Winograd. Nick and Barbara Garrison are close personal friends and our bicycle companions on weekends and trips. Summers have been highlighted by boating with the Schimmels and Kozarichs. Xin Zhang, now an associate professor, and I have an exciting collaboration featuring his ingenious fluorescent probes to report on the composition of cellular interiors. Particularly gratifying is the promise of a joint research effort with Paul Mischel (University of California, San Diego) on aggressive tumors that may be fueled by the purinosome the lab has been studying.

Based on the lab's publications and reputation, I had the opportunity to serve the scientific community through participation in NIH study sections and Howard Hughes Medical Institute panels and as a Reviewing Editor for *Biochemistry*, *Proceedings of the National Academy of Sciences*, and *Science*, the last for 28 years. I have been honored by invitations to give numerous lectures, appointments to academies, and a variety of awards—one of the more enjoyable included a meeting with President Obama.

My career has been a remarkable odyssey: challenging but never boring, enlivened by many collaborators and gifted graduate students and postdoctoral fellows, and broadened outside of academia by consulting and entrepreneurial ventures. None of my success would have been achieved without my closest friend, confidante, advisor, and critic: Pat.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Throughout the text, I have named several of my graduate students and postdoctoral fellows who made significant contributions to the lab's research success. Most of the references are taken from some 630 papers my lab has published. These are among the more highly cited papers, and the graduate students and postdoctoral fellows involved have gone on to hold positions at universities such as Arkansas, Baylor, Brandeis, Cambridge, Cape Town, Case Western, Centre National de la Recherche Scientifique (CNRS), Colorado, Delaware, Drexel, Emory, Florida, Iowa, Iowa State, Johns Hopkins, Leeds, Lehigh, Maryland, Michigan, Minnesota, Montclair, North Carolina, Nottingham, Purdue, Stonehill, Temple, Texas, Thomas Jefferson, the Korean Academy of Science and Technology, UT Southwestern, Waterloo, and Wayne and companies such as Corning, DuPont, Eli Lilly, Genentech, GlaxoSmithKline, Pfizer, and Rohm Haas. These are by no means exhaustive listings.

LITERATURE CITED

1. Fruton JS, Simmonds S. 1958. *General Biochemistry*. New York: Wiley. 2nd ed.
2. Sumner JB. 1926. The isolation and crystallization of the enzyme urease. *J. Biol. Chem.* 69:435–41
3. Neurath H, Hartley BS. 1959. The hydrolysis of peptide and ester bonds by proteolytic enzymes. *J. Cell. Comp. Physiol.* 54:179–202
4. Bruice TC, Benkovic SJ, French TC. 1965. Stopped-flow apparatus for a Zeiss PMQ II spectrophotometer. *Rev. Sci. Inst.* 36:860–61

5. Bruice TC, Benkovic SJ. 1963. A Comparison of the bimolecular and intramolecular nucleophilic catalysis of the hydrolysis of substituted phenyl acetates by the dimethylamino group. *J. Am. Chem. Soc.* 85:1–8
6. Bruice TC, Benkovic SJ. 1966. *Bioorganic Mechanisms, Vol. I*. New York: Benjamin
7. Bruice TC, Benkovic SJ. 1966. *Bioorganic Mechanisms, Vol. II*. New York: Benjamin
8. Benkovic SJ, Schray KJ. 1968. The kinetics and mechanisms of phosphoenolpyruvate hydrolysis. *Biochemistry* 7:4090–96
9. Schray KJ, Benkovic SJ. 1971. Mechanisms of hydrolysis of phosphate ester derivatives of phosphoenolpyruvic acid. *J. Am. Chem. Soc.* 93:2522–29
10. Benkovic SJ, Benkovic PA, Chrzanowski R. 1970. Studies on models for tetrahydrofolic acid. II. The mechanism for condensation of formaldehyde with tetrahydroquinoxaline analogs. *J. Am. Chem. Soc.* 92:523–28
11. Benkovic SJ, Bullard WP, Benkovic PA. 1972. Studies on models for tetrahydrofolic acid. III. Hydrolytic interconversions of the tetrahydroquinoxaline analogs at the formate level of oxidation. *J. Am. Chem. Soc.* 94:7542–49
12. Benkovic SJ. 1966. Studies on sulfate esters. II. Carboxyl group catalysis in the hydrolysis of salicyl sulfate. *J. Am. Chem. Soc.* 88:5511–15
13. Benkovic SJ, Benkovic PA. 1968. Studies on sulfate esters. III. A comparison of the solvolyses of salicyl sulfate and sulfur trioxide. *J. Am. Chem. Soc.* 90:2646–50
14. Benkovic SJ, Kleinschuster JJ, deMaine MM, Siewers IJ. 1971. On the mechanism of action of fructose 1,6-diphosphatase. Inhibition by structural analogs of fructose 1,6-diphosphate. *Biochemistry* 10:4881–87
15. Libby CB, Frey WA, Villafranca JJ, Benkovic SJ. 1975. Kinetic and binding studies of Mn (II) and fructose 1,6-bisphosphate with rabbit liver hexosebisphosphatase. *J. Biol. Chem.* 250:7564–73
16. Schray KJ, Benkovic SJ, Benkovic PA, Rose IA. 1973. Catalytic reactions of phosphoglucose isomerase with cyclic forms of glucose 6-phosphate and fructose 6-phosphate. *J. Biol. Chem.* 248:2219–24
17. Fishbein R, Benkovic PA, Schray KJ, Siewers IJ, Steffens JJ, Benkovic SJ. 1974. Anomeric specificity of phosphofructokinase from rabbit muscle. *J. Biol. Chem.* 249:6047–51
18. Schray KJ, Fishbein R, Bullard WP, Benkovic SJ. 1975. The anomeric form of D-fructose 1,6-bisphosphate used as substrate in the muscle and yeast aldolase reactions. *J. Biol. Chem.* 250:4883–87
19. Frey WA, Fishbein R, deMaine MM, Benkovic SJ. 1977. Substrate form of D-fructose 1,6-bisphosphate utilized by fructose 1,6-bisphosphatase. *Biochemistry* 16:2479–84
20. Benkovic SJ. 1979. Anomeric specificity of carbohydrate-utilizing enzymes. *Methods Enzymol.* 63:370–79
21. Smith GK, Mueller WT, Wasserman GF, Taylor WD, Benkovic SJ. 1980. Characterization of the enzyme complex involving the folate-requiring enzymes of de novo purine biosynthesis. *Biochemistry* 19:4313–21
22. Caperelli CA, Benkovic PA, Chettur G, Benkovic SJ. 1980. Purification of a complex catalyzing folate cofactor synthesis and transformylation in de novo purine biosynthesis. *J. Biol. Chem.* 255:1885–90
23. Smith GK, Mueller WT, Benkovic PA, Benkovic SJ. 1981. On the cofactor specificity of glycinamide ribonucleotide and 5-aminoimidazole-4-carboxamide ribonucleotide transformylase from chicken liver. *Biochemistry* 20:1241–45
24. Inglese J, Smith JM, Benkovic SJ. 1990. Active-site mapping and site-specific mutagenesis of glycinamide ribonucleotide transformylase from *Escherichia coli*. *Biochemistry* 29:6678–87
25. Inglese J, Benkovic SJ. 1991. Multisubstrate adduct inhibitors of glycinamide ribonucleotide transformylase: synthetic and enzyme-assembled. *Tetrahedron Lett.* 47:2351–64
26. Moad G, Luthy CL, Benkovic SJ. 1978. The mechanism of oxidation of 6-methyl-5-carba-5-deazatetrahydropterin. Evidence for the involvement of a 4a-adduct in the oxidation of tetrahydropterins. *Tetrahedron Lett.* 19:2271–74
27. Lazarus RA, Dietrich RF, Wallick DE, Benkovic SJ. 1981. On the mechanism of action of phenylalanine hydroxylase. *Biochemistry* 20:6834–41
28. Lazarus RA, DeBrosse CW, Benkovic SJ. 1982. Phenylalanine hydroxylase: structural determination of the tetrahydropterin intermediates by ^{13}C NMR spectroscopy. *J. Am. Chem. Soc.* 104:6869–71
29. Gottschall DW, Dietrich RF, Benkovic SJ, Shiman R. 1982. Phenylalanine hydroxylase. Correlation of the iron content with activity and the preparation and reconstitution of the apoenzyme. *J. Biol. Chem.* 257:845–49

30. Benkovic SJ, Sammons D, Armarego WLF, Waring P, Inners R. 1985. Tautomeric nature of quinonoid 6,7-dimethyl-7,8-dihydro-6*H*-pterin in aqueous solution: a ^{15}N NMR study. *J. Am. Chem. Soc.* 107:3706–12
31. Carr RT, Balasubramanian S, Hawkins PC, Benkovic SJ. 1995. Mechanism of metal-independent hydroxylation by *Chromobacterium violaceum* phenylalanine hydroxylase. *Biochemistry* 34:7525–32
32. Shim JH, Benkovic SJ. 1998. Evaluation of the kinetic mechanism of *Escherichia coli* glycinamide ribonucleotide transformylase. *Biochemistry* 37:8776–82
33. Smith GK, Benkovic PA, Benkovic SJ. 1981. L(−)-10-Formyltetrahydrofolate is the cofactor for glycinamide ribonucleotide transformylase from chicken liver. *Biochemistry* 20:4034–36
34. Smith GK, Mueller WT, Slieker LJ, DeBrosse CW, Benkovic SJ. 1982. Direct transfer of one-carbon units in the transformylations of de novo purine biosynthesis. *Biochemistry* 21:2870–74
35. Daubner SC, Schrimsher JL, Schendel FJ, Young M, Henikoff S, et al. 1985. A multifunctional protein possessing glycinamide ribonucleotide synthetase, glycinamide ribonucleotide transformylase, and aminoimidazole ribonucleotide synthetase activities in de novo purine biosynthesis. *Biochemistry* 24:7059–62
36. Lazarus RA, Benkovic SJ, Kaufman S. 1983. Phenylalanine hydroxylase stimulator protein is a 4*a*-carbinolamine dehydratase. *J. Biol. Chem.* 258:10960–62
37. Pember SO, Villafranca JJ, Benkovic SJ. 1986. Phenylalanine hydroxylase from *Chromobacterium violaceum* is a copper-containing monooxygenase. Kinetics of the reductive activation of the enzyme. *Biochemistry* 25:6611–19
38. Marlier JF, Bryant FR, Benkovic SJ. 1981. Stereochemical and kinetic investigation of ^{32}P-labeled inorganic phosphate exchange reaction catalyzed by primer-independent and primer-dependent polynucleotide phosphorylase from *Micrococcus luteus*. *Biochemistry* 20:2212–19
39. Bryant FR, Benkovic SJ, Sammons D, Frey PA. 1981. The stereochemical course of thiophosphoryl group transfer catalyzed by T4 polynucleotide kinase. *J. Biol. Chem.* 256:5965–66
40. Rahil JF, deMaine MM, Benkovic SJ. 1982. Rapid-quench and isotope-trapping studies on fructose-1,6-bisphosphatase. *Biochemistry* 21:3358–63
41. Bryant FR, Johnson KA, Benkovic SJ. 1983. Elementary steps in the DNA polymerase I reaction pathway. *Biochemistry* 22:3537–46
42. Gupta AP, Benkovic SJ. 1984. Stereochemical course of the 3′–5′-exonuclease activity of DNA polymerase I. *Biochemistry* 23:5874–81
43. Slieker LJ, Benkovic SJ. 1984. Synthesis of (6*R*,11*S*)- and (6*R*,11*R*)-5,10-methylene[11–^{1}H,^{2}H]tetrahydrofolate. Stereochemical paths of serine hydroxymethyltransferase, 5,10-methylenetetrahydrofolate dehydrogenase, and thymidylate synthetase catalysis. *J. Am. Chem. Soc.* 106:1833–38
44. Domanico PL, Rahil JF, Benkovic SJ. 1985. Unambiguous stereochemical course of rabbit liver fructose bisphosphatase hydrolysis. *Biochemistry* 24:1623–28
45. Mizrahi V, Benkovic P, Benkovic SJ. 1986. Mechanism of DNA polymerase I: exonuclease/polymerase activity switch and DNA sequence dependence of pyrophosphorolysis and misincorporation reactions. *PNAS* 83:5769–73
46. Mizrahi V, Benkovic PA, Benkovic SJ. 1986. Mechanism of the idling-turnover reaction of the large (Klenow) fragment of *Escherichia coli* DNA polymerase I. *PNAS* 83:231–35
47. Kuchta RD, Mizrahi V, Benkovic PA, Johnson KA, Benkovic SJ. 1987. Kinetic mechanism of DNA polymerase I (Klenow). *Biochemistry* 26:8410–17
48. Kuchta RD, Benkovic P, Benkovic SJ. 1988. Kinetic mechanism whereby DNA polymerase I (Klenow) replicates DNA with high fidelity. *Biochemistry* 27:6716–25
49. Allen DJ, Benkovic SJ. 1989. Resonance energy transfer measurements between substrate binding sites within the large (Klenow) fragment of *Escherichia coli* DNA polymerase I. *Biochemistry* 28:9586–93
50. Guest CR, Hochstrasser RA, Dupuy CG, Allen DJ, Benkovic SJ, Millar DP. 1991. Interaction of DNA with the Klenow fragment of DNA polymerase I studied by time-resolved fluorescence spectroscopy. *Biochemistry* 30:8759–70

51. Dahlberg ME, Benkovic SJ. 1991. Kinetic mechanism of DNA polymerase I (Klenow fragment): identification of a second conformational change and evaluation of the internal equilibrium constant. *Biochemistry* 30:4835–43
52. Alberts BM, Barry J, Bedinger P, Formosa T, Jongeneel CV, Kreuzer KN. 1983. Studies on DNA replication in the bacteriophage T4 in vitro system. *Cold Spring Harb. Symp. Quant. Biol.* 47(Part 2):655–68
53. Alberts BM. 1985. Protein machines mediate the basic genetic processes. *Trends Genet.* 1:26–30
54. Nossal NG. 1992. Protein-protein interactions at a DNA replication fork: bacteriophage T4 as a model. *FASEB J.* 6(3):871–78
55. Capson TL, Benkovic SJ, Nossal NG. 1991. Protein-DNA cross-linking demonstrates stepwise ATP-dependent assembly of T4 DNA polymerase and its accessory proteins on the primer-template. *Cell* 65:249–58
56. Capson TL, Peliska JA, Kaboord BF, Frey MW, Lively C, et al. 1992. Kinetic characterization of the polymerase and exonuclease activities of the gene 43 protein of bacteriophage T4. *Biochemistry* 31:10984–94
57. Kaboord BF, Benkovic SJ. 1993. Rapid assembly of the bacteriophage T4 core replication complex on a linear primer/template construct. *PNAS* 90:10881–85
58. Kaboord BF, Benkovic SJ. 1995. Accessory proteins function as matchmakers in the assembly of the T4 DNA polymerase holoenzyme. *Curr. Biol.* 5:149–57
59. Kaboord BF, Benkovic SJ. 1996. Dual role of the 44/62 protein as a matchmaker protein and DNA polymerase chaperone during assembly of the bacteriophage T4 holoenzyme complex. *Biochemistry* 35:1084–92
60. Berdis AJ, Benkovic SJ. 1996. Role of adenosine 5′-triphosphate hydrolysis in the assembly of the bacteriophage T4 DNA replication holoenzyme complex. *Biochemistry* 35:9253–65
61. Sexton DJ, Berdis AJ, Benkovic SJ. 1997. Assembly and disassembly of DNA polymerase holoenzyme. *Curr. Opin. Chem. Biol.* 1:316–22
62. Carver TE Jr., Sexton DJ, Benkovic SJ. 1997. Dissociation of bacteriophage T4 DNA polymerase and its processivity clamp after completion of Okazaki fragment synthesis. *Biochemistry* 36:14409–17
63. Berdis AJ, Benkovic SJ. 1997. Mechanism of bacteriophage T4 DNA holoenzyme assembly: the 44/62 protein acts as a molecular motor. *Biochemistry* 36:2733–43
64. Soumillion P, Sexton DJ, Benkovic SJ. 1998. Clamp subunit dissociation dictates bacteriophage T4 DNA polymerase holoenzyme disassembly. *Biochemistry* 37:1819–27
65. Sexton DJ, Kaboord BF, Berdis AJ, Carver TE, Benkovic SJ. 1998. Dissecting the order of bacteriophage T4 DNA polymerase holoenzyme assembly. *Biochemistry* 37:7749–56
66. Kroutil LC, Frey MW, Kaboord BF, Kunkel TA, Benkovic SJ. 1998. Effect of accessory proteins on T4 DNA polymerase replication fidelity. *J. Mol. Biol.* 278:135–46
67. Berdis AJ, Benkovic SJ. 1998. Simultaneous formation of functional leading and lagging strand holoenzyme complexes on a small, defined DNA substrate. *PNAS* 95:11128–33
68. Alley SC, Ishmael FT, Jones AD, Benkovic SJ. 2000. Mapping protein–protein interactions in the bacteriophage T4 DNA polymerase holoenzyme using a novel trifunctional photo-cross-linking and affinity reagent. *J. Am. Chem. Soc.* 122:6126–27
69. Alley SC, Abel-Santos E, Benkovic SJ. 2000. Tracking sliding clamp opening and closing during bacteriophage T4 DNA polymerase holoenzyme assembly. *Biochemistry* 39:3076–90
70. Valentine AM, Ishmael FT, Shier VK, Benkovic SJ. 2001. A zinc ribbon protein in DNA replication: primer synthesis and macromolecular interactions by the bacteriophage T4 primase. *Biochemistry* 40:15074–85
71. Alley SC, Trakselis MA, Mayer MU, Ishmael FT, Jones AD, Benkovic SJ. 2001. Building a replisome solution structure by elucidation of protein-protein interactions in the bacteriophage T4 DNA polymerase holoenzyme. *J. Biol. Chem.* 276:39340–49
72. Trakselis MA, Alley SC, Abel-Santos E, Benkovic SJ. 2001. Creating a dynamic picture of the sliding clamp during T4 DNA polymerase holoenzyme assembly by using fluorescence resonance energy transfer. *PNAS* 98:8368–75
73. Trakselis MA, Mayer MU, Ishmael FT, Roccasecca RM, Benkovic SJ. 2001. Dynamic protein interactions in the bacteriophage T4 replisome. *Trends Biochem. Sci.* 26:566–72

74. Trakselis MA, Berdis AJ, Benkovic SJ. 2003. Examination of the role of the clamp-loader and ATP hydrolysis in the formation of the bacteriophage T4 polymerase holoenzyme. *J. Mol. Biol.* 326:435–51
75. Millar D, Trakselis MA, Benkovic SJ. 2004. On the solution structure of the T4 sliding clamp (gp45). *Biochemistry* 43:12723–27
76. Yang J, Zhuang Z, Roccasecca RM, Trakselis MA, Benkovic SJ. 2004. The dynamic processivity of the T4 DNA polymerase during replication. *PNAS* 101:8289–94
77. Zhang Z, Spiering MM, Trakselis MA, Ishmael FT, Xi J, et al. 2005. Assembly of the bacteriophage T4 primosome: single-molecule and ensemble studies. *PNAS* 102:3254–59
78. Wong KF, Selzer T, Benkovic SJ, Hammes-Schiffer S. 2005. Impact of distal mutations on the network of coupled motions correlated to hydride transfer in dihydrofolate reductase. *PNAS* 102:6807–12
79. Xi J, Zhang Z, Zhuang Z, Yang J, Spiering MM, et al. 2005. Interaction between the T4 helicase loading protein (gp59) and the DNA polymerase (gp43): unlocking of the gp59-gp43-DNA complex to initiate assembly of a fully functional replisome. *Biochemistry* 44:7747–56
80. Xi J, Zhuang Z, Zhang Z, Selzer T, Spiering MM, et al. 2005. Interaction between the T4 helicase-loading protein (gp59) and the DNA polymerase (gp43): a locking mechanism to delay replication during replisome assembly. *Biochemistry* 44:2305–18
81. Smiley RD, Zhuang Z, Benkovic SJ, Hammes GG. 2006. Single-molecule investigation of the T4 bacteriophage DNA polymerase holoenzyme: multiple pathways of holoenzyme formation. *Biochemistry* 45:7990–97
82. Nelson SW, Yang J, Benkovic SJ. 2006. Site-directed mutations of T4 helicase loading protein (gp59) reveal multiple modes of DNA polymerase inhibition and the mechanism of unlocking by gp41 helicase. *J. Biol. Chem.* 281:8697–706
83. Yang J, Nelson SW, Benkovic SJ. 2006. The control mechanism for lagging strand polymerase recycling during bacteriophage T4 DNA replication. *Mol. Cell* 21:153–64
84. Lionnet T, Spiering MM, Benkovic SJ, Bensimon D, Croquette V. 2007. Real-time observation of bacteriophage T4 gp41 helicase reveals an unwinding mechanism. *PNAS* 104:19790–95
85. Nelson SW, Kumar R, Benkovic SJ. 2008. RNA primer handoff in bacteriophage T4 DNA replication: the role of single-stranded DNA-binding protein and polymerase accessory proteins. *J. Biol. Chem.* 283:22838–46
86. Arumugam SR, Lee TH, Benkovic SJ. 2009. Investigation of stoichiometry of T4 bacteriophage helicase loader protein (gp59). *J. Biol. Chem.* 284:29283–89
87. Manosas M, Spiering MM, Zhuang Z, Benkovic SJ, Croquette V. 2009. Coupling DNA unwinding activity with primer synthesis in the bacteriophage T4 primosome. *Nat. Chem. Biol.* 5:904–12
88. Manosas M, Perumal SK, Croquette V, Benkovic SJ. 2012. Direct observation of stalled fork restart via fork regression in the T4 replication system. *Science* 338:1217–20
89. Manosas M, Spiering MM, Ding F, Bensimon D, Allemand JF, et al. 2012. Mechanism of strand displacement synthesis by DNA replicative polymerases. *Nucleic Acids Res.* 40:6174–86
90. Hedglin M, Perumal SK, Hu Z, Benkovic S. 2013. Stepwise assembly of the human replicative polymerase holoenzyme. *eLife* 2:e00278
91. Spiering MM, Hanoian P, Gannavaram S, Benkovic SJ. 2017. RNA primer-primase complexes serve as the signal for polymerase recycling and Okazaki fragment initiation in T4 phage DNA replication. *PNAS* 114:5635–40
92. Benkovic SJ, Spiering MM. 2017. Understanding DNA replication by the bacteriophage T4 replisome. *J. Biol. Chem.* 292:18434–42
93. Matthews DA, Bolin JT, Burridge JM, Filman DJ, Volz KW, et al. 1985. Refined crystal structures of *Escherichia coli* and chicken liver dihydrofolate reductase containing bound trimethoprim. *J. Biol. Chem.* 260:381–91
94. Iyengar R, Rose IA. 1981. Liberation of the triosephosphate isomerase reaction intermediate and its trapping by isomerase, yeast aldolase, and methylglyoxal synthase. *Biochemistry* 20:1229–35
95. Pompliano DL, Peyman A, Knowles JR. 1990. Stabilization of a reaction intermediate as a catalytic device: definition of the functional role of the flexible loop in triosephosphate isomerase. *Biochemistry* 29:3186–94

96. Fierke CA, Johnson KA, Benkovic SJ. 1987. Construction and evaluation of the kinetic scheme associated with dihydrofolate reductase from *Escherichia coli*. *Biochemistry* 26:4085–92
97. Parvez M, Napper AD, Benkovic SJ. 1988. Structure of 6-(iodomethyl)-2-oxo-2-phenoxy-1,2-oxaphosphorinane. *Acta Crystallogr. C* 44(Part 8):1414–16
98. Adams J, Johnson K, Matthews R, Benkovic SJ. 1989. Effects of distal point-site mutations on the binding and catalysis of dihydrofolate reductase from *Escherichia coli*. *Biochemistry* 28:6611–18
99. Fierke CA, Benkovic SJ. 1989. Probing the functional role of threonine-113 of *Escherichia coli* dihydrofolate reductase for its effect on turnover efficiency, catalysis, and binding. *Biochemistry* 28:478–86
100. Li LY, Benkovic SJ. 1991. Impact on catalysis of secondary structural manipulation of the αC-helix of *Escherichia coli* dihydrofolate reductase. *Biochemistry* 30:1470–78
101. Adams JA, Fierke CA, Benkovic SJ. 1991. The function of amino acid residues contacting the nicotinamide ring of NADPH in dihydrofolate reductase from *Escherichia coli*. *Biochemistry* 30:11046–54
102. Wagner CR, Thillet J, Benkovic SJ. 1992. Complementary perturbation of the kinetic mechanism and catalytic effectiveness of dihydrofolate reductase by side-chain interchange. *Biochemistry* 31:7834–40
103. Wagner CR, Huang Z, Singleton SF, Benkovic SJ. 1995. Molecular basis for nonadditive mutational effects in *Escherichia coli* dihydrofolate reductase. *Biochemistry* 34:15671–80
104. Cameron CE, Benkovic SJ. 1997. Evidence for a functional role of the dynamics of glycine-121 of *Escherichia coli* dihydrofolate reductase obtained from kinetic analysis of a site-directed mutant. *Biochemistry* 36:15792–800
105. Rajagopalan PT, Lutz S, Benkovic SJ. 2002. Coupling interactions of distal residues enhance dihydrofolate reductase catalysis: mutational effects on hydride transfer rates. *Biochemistry* 41:12618–28
106. Antikainen NM, Smiley RD, Benkovic SJ, Hammes GG. 2005. Conformation coupled enzyme catalysis: single-molecule and transient kinetics investigation of dihydrofolate reductase. *Biochemistry* 44:16835–43
107. Wang L, Tharp S, Selzer T, Benkovic SJ, Kohen A. 2006. Effects of a distal mutation on active site chemistry. *Biochemistry* 45:1383–92
108. Janda KD, Schloeder D, Benkovic SJ, Lerner RA. 1988. Induction of an antibody that catalyzes the hydrolysis of an amide bond. *Science* 241:1188–91
109. Pollack SJ, Jacobs JW, Schultz PG. 1986. Selective chemical catalysis by an antibody. *Science* 234:1570–73
110. Napper AD, Benkovic SJ, Tramontano A, Lerner RA. 1987. A stereospecific cyclization catalyzed by an antibody. *Science* 237:1041–43
111. Lerner RA, Benkovic SJ. 1988. Principles of antibody catalysis. *BioEssays* 9:107–12
112. Janda KD, Benkovic SJ, Lerner RA. 1989. Catalytic antibodies with lipase activity and R or S substrate selectivity. *Science* 244:437–40
113. Janda KD, Weinhouse MI, Schloeder DM, Benkovic SJ, Lerner RA. 1990. Bait and switch strategy for obtaining catalytic antibodies with acyl-transfer capabilities. *J. Am. Chem. Soc.* 112:1274–75
114. Iverson BL, Iverson SA, Roberts VA, Getzoff ED, Tainer JA, et al. 1990. Metalloantibodies. *Science* 249:659–62
115. Benkovic SJ, Adams JA, Borders CL Jr., Janda KD, Lerner RA. 1990. The enzymic nature of antibody catalysis: development of multistep kinetic processing. *Science* 250:1135–39
116. Lerner RA, Benkovic SJ, Schultz PG. 1991. At the crossroads of chemistry and immunology: catalytic antibodies. *Science* 252:659–67
117. Janda KD, Ashley JA, Jones TM, McLeod DA, Schloeder DM, et al. 1991. Catalytic antibodies with acyl-transfer capabilities: mechanistic and kinetic investigations. *J. Am. Chem. Soc.* 113:291–97
118. Gibbs RA, Posner BA, Filpula DR, Dodd SW, Finkelman MA, et al. 1991. Construction and characterization of a single-chain catalytic antibody. *PNAS* 88:4001–4
119. Benkovic SJ. 1992. Catalytic antibodies. *Annu. Rev. Biochem.* 61:29–54
120. Huse WD, Sastry L, Iverson SA, Kang AS, Alting-Mees M, et al. 1989. Generation of a large combinatorial library of the immunoglobulin repertoire in phage lambda. *Science* 246:1275–81
121. Gibbs RA, Benkovic PA, Janda KD, Lerner RA, Benkovic SJ. 1992. Substituent effects of an antibody-catalyzed hydrolysis of phenyl esters: further evidence for an acyl-antibody intermediate. *J. Am. Chem. Soc.* 114:3528–34

122. Stewart JD, Benkovic SJ. 1993. Catalytic antibodies: mechanistic and practical considerations. *Chem. Soc. Rev.* 22:213–19
123. Stewart JD, Krebs JF, Siuzdak G, Berdis AJ, Smithrud DB, Benkovic SJ. 1994. Dissection of an antibody-catalyzed reaction. *PNAS* 91:7404–9
124. Hirschmann R, Smith AB 3rd, Taylor CM, Benkovic PA, Taylor SD, et al. 1994. Peptide synthesis catalyzed by an antibody containing a binding site for variable amino acids. *Science* 265:234–37
125. Smiley JA, Benkovic SJ. 1994. Selection of catalytic antibodies for a biosynthetic reaction from a combinatorial cDNA library by complementation of an auxotrophic *Escherichia coli*: antibodies for orotate decarboxylation. *PNAS* 91:8319–23
126. Stewart JD, Benkovic SJ. 1995. Transition-state stabilization as a measure of the efficiency of antibody catalysis. *Nature* 375:388–91
127. Smithrud DB, Benkovic PA, Benkovic SJ, Roberts V, Liu J, et al. 2000. Cyclic peptide formation catalyzed by an antibody ligase. *PNAS* 97:1953–58
128. Barbas CF 3rd, Kang AS, Lerner RA, Benkovic SJ. 1991. Assembly of combinatorial antibody libraries on phage surfaces: the gene III site. *PNAS* 88:7978–82
129. Kang AS, Barbas CF, Janda KD, Benkovic SJ, Lerner RA. 1991. Linkage of recognition and replication functions by assembling combinatorial antibody Fab libraries along phage surfaces. *PNAS* 88:4363–66
130. Stewart JD, Benkovic SJ. 1993. Reaction mechanisms displayed by catalytic antibodies. *Acc. Chem. Res.* 10:396–404
131. Falzone CJ, Benkovic SJ, Wright PE. 1990. Partial ^{1}H NMR assignments of the *Escherichia coli* dihydrofolate reductase complex with folate: evidence for a unique conformation of bound folate. *Biochemistry* 29:9667–77
132. Falzone CJ, Cavanagh J, Cowart M, Palmer AG 3rd, Matthews CR, et al. 1994. ^{1}H, ^{15}N and ^{13}C resonance assignments, secondary structure, and the conformation of substrate in the binary folate complex of *Escherichia coli* dihydrofolate reductase. *J. Biomol. NMR* 4:349–66
133. Epstein DM, Benkovic SJ, Wright PE. 1995. Dynamics of the dihydrofolate reductase-folate complex: Catalytic sites and regions known to undergo conformational change exhibit diverse dynamical features. *Biochemistry* 34:11037–48
134. Bhabha G, Lee J, Ekiert DC, Gam J, Wilson IA, et al. 2011. A dynamic knockout reveals that conformational fluctuations influence the chemical step of enzyme catalysis. *Science* 332:234–38
135. Liu CT, Benkovic SJ. 2013. Capturing a sulfenic acid with arylboronic acids and benzoxaborole. *J. Am. Chem. Soc.* 135:14544–47
136. Cannon WR, Singleton SF, Benkovic SJ. 1996. A perspective on biological catalysis. *Nat. Struct. Biol.* 3:821–33
137. Cannon WR, Benkovic SJ. 1998. Solvation, reorganization energy, and biological catalysis. *J. Biol. Chem.* 273:26257–60
138. Hammes-Schiffer S, Benkovic SJ. 2006. Relating protein motion to catalysis. *Annu. Rev. Biochem.* 75:519–41
139. Benkovic SJ, Hammes-Schiffer S. 2007. Dihydrofolate reductase: hydrogen tunneling and protein motion. In *Hydrogen-Transfer Reactions, Vol.* 4, ed. JT Hynes, RL Schowen, JP Klinman, H-H Limbach, pp. 1439–54. Weinheim, Ger.: Wiley-VCH Verlag
140. Agarwal PK, Billeter SR, Rajagopalan PT, Benkovic SJ, Hammes-Schiffer S. 2002. Network of coupled promoting motions in enzyme catalysis. *PNAS* 99:2794–99
141. Benkovic SJ, Hammes-Schiffer S. 2003. A perspective on enzyme catalysis. *Science* 301:1196–202
142. Hammes-Schiffer S, Benkovic SJ. 2006. Relating protein motion to catalysis. *Annu. Rev. Biochem.* 75:519–41
143. Nashine VC, Hammes-Schiffer S, Benkovic SJ. 2010. Coupled motions in enzyme catalysis. *Curr. Opin. Chem. Biol.* 14:644–51
144. Hammes GG, Benkovic SJ, Hammes-Schiffer S. 2011. Flexibility, diversity, and cooperativity: pillars of enzyme catalysis. *Biochemistry* 50:10422–30
145. Liu CT, Layfield JP, Stewart RJ 3rd, French JB, Hanoian P, et al. 2014. Probing the electrostatics of active site microenvironments along the catalytic cycle for *Escherichia coli* dihydrofolate reductase. *J. Am. Chem. Soc.* 136:10349–60

146. Hanoian P, Liu CT, Hammes-Schiffer S, Benkovic S. 2015. Perspectives on electrostatics and conformational motions in enzyme catalysis. *Acc. Chem. Res.* 48:482–89
147. Boger DL, Haynes NE, Warren MS, Ramcharan J, Kitos PA, Benkovic SJ. 1997. Multisubstrate analogue based on 5,8,10-trideazafolate. *Bioorg. Med. Chem.* 5:1853–57
148. Su Y, Yamashita MM, Greasley SE, Mullen CA, Shim JH, et al. 1998. A pH-dependent stabilization of an active site loop observed from low and high pH crystal structures of mutant monomeric glycinamide ribonucleotide transformylase at 1.8 to 1.9 Å. *J. Mol. Biol.* 281:485–99
149. Boger DL, Kochanny MJ, Cai H, Wyatt D, Kitos PA, et al. 1998. Design, synthesis, and evaluation of potential GAR and AICAR transformylase inhibitors. *Bioorg. Med. Chem.* 6:643–59
150. Greasley SE, Yamashita MM, Cai H, Benkovic SJ, Boger DL, Wilson IA. 1999. New insights into inhibitor design from the crystal structure and NMR studies of *Escherichia coli* GAR transformylase in complex with β-GAR and 10-formyl-5,8,10-trideazafolic acid. *Biochemistry* 38:16783–93
151. Marsilje TH, Hedrick MP, Desharnais J, Tavassoli A, Zhang Y, et al. 2003. Design, synthesis, and biological evaluation of simplified α-keto heterocycle, trifluoromethyl ketone, and formyl substituted folate analogues as potential inhibitors of GAR transformylase and AICAR transformylase. *Bioorg. Med. Chem.* 11:4487–501
152. Marsilje TH, Hedrick MP, Desharnais J, Capps K, Tavassoli A, et al. 2003. 10-(2-benzoxazolcarbonyl)-5,10-dideaza-acyclic-5,6,7,8-tetrahydrofolic acid: a potential inhibitor of GAR transformylase and AICAR transformylase. *Bioorg. Med. Chem.* 11:4503–9
153. Zhang Y, Desharnais J, Marsilje TH, Li C, Hedrick MP, et al. 2003. Rational design, synthesis, evaluation, and crystal structure of a potent inhibitor of human GAR Tfase: 10-(trifluoroacetyl)-5,10-dideazaacyclic-5,6,7,8-tetrahydrofolic acid. *Biochemistry* 42:6043–56
154. Wolan DW, Greasley SE, Wall MJ, Benkovic SJ, Wilson IA. 2003. Structure of avian AICAR transformylase with a multisubstrate adduct inhibitor β-DADF identifies the folate binding site. *Biochemistry* 42:10904–14
155. An S, Kumar R, Sheets ED, Benkovic SJ. 2008. Reversible compartmentalization of de novo purine biosynthetic complexes in living cells. *Science* 320:103–6
156. An S, Deng Y, Tomsho JW, Kyoung M, Benkovic SJ. 2010. Microtubule-assisted mechanism for functional metabolic macromolecular complex formation. *PNAS* 107:12872–76
157. French JB, Zhao H, An S, Niessen S, Deng Y, et al. 2013. Hsp70/Hsp90 chaperone machinery is involved in the assembly of the purinosome. *PNAS* 110:2528–33
158. French JB, Jones SA, Deng H, Pedley AM, Kim D, et al. 2016. Spatial colocalization and functional link of purinosomes with mitochondria. *Science* 351:733–37
159. Pedley AM, Benkovic SJ. 2017. A new view into the regulation of purine metabolism: the purinosome. *Trends Biochem. Sci.* 42:141–54
160. Benkovic SJ, Pedley AM, Spiering MM. 2018. Intracellular enzyme organization. *SciTech Eur. Q.* 26:1–2
161. Pareek V, Tian H, Winograd N, Benkovic SJ. 2020. Metabolomics and mass spectrometry imaging reveal channeled de novo purine synthesis in cells. *Science* 368:283–90
162. Pedley AM, Benkovic SJ. 2018. Detecting purinosome metabolon formation with fluorescence microscopy. *Methods Mol. Biol.* 1764:279–89