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Inspired *Beyond* Nature: Three Decades of Spherical Nucleic Acids and Colloidal Crystal Engineering with DNA

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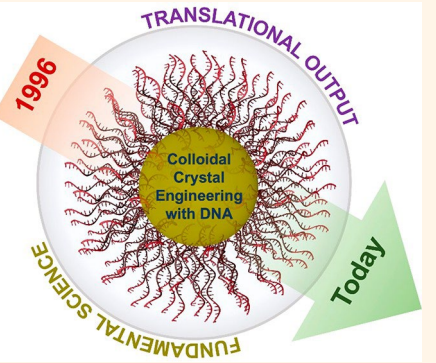
ABSTRACT: The conception, synthesis, and invention of a nanostructure, now known as the spherical nucleic acid, or SNA, in 1996 marked the advent of a new field of chemistry. Over the past three decades, the SNA and its analogous anisotropic equivalents have provided an avenue for us to think about some of the most fundamental concepts in chemistry in new ways and led to technologies that are significantly impacting fields from medicine to materials science. A prime example is colloidal crystal engineering with DNA, the framework for using SNAs and related structures to synthesize programmable matter. Herein, we document the evolution of this framework, which was initially inspired *by* nature, and describe how it now allows researchers to chart paths to move *beyond* it, as programmable matter with real-world significance is envisioned and created.



1. COLLOIDAL CRYSTAL ENGINEERING WITH DNA: INTRODUCTION AND IMPACT

Nature has been the ultimate programmer of matter.[1] Over billions of years, life on Earth has been orchestrated in increasing levels of complexity via chemical and biological processes that have evolved over time. Atoms form molecules, molecules interact with each other to become larger assemblies of molecules, and ultimately these larger assemblies become the macroscale objects that we see around us. The world is a collection of different types of matter, all constructed from the atoms that comprise the Periodic Table of the Elements, the fundamental building blocks of the natural world. Nature has the rules for making materials baked in, and atomic properties are fixed.

For centuries, chemists have been learning the rules that govern natural phenomena, and we have made extraordinary progress.[1] We have figured out what the atoms are, what properties they have, and how they can be combined using chemical reactions. But, in many ways, although scientists have been inspired by nature, in fact, often seeking to mimic it, they have been at its mercy, limited by the combinations of elements with their fixed electronic configurations that are compatible. That was then. We have moved into a new era, where, as chemists, we are not simply inspired *by* nature but rather empowered to move *beyond* it. Today, chemists are writing the rules (Table 1) to build matter at the nanoscale, many examples of which have properties that have never been observed in natural systems.

Researchers, using nanoparticles as elementary building blocks, have devised ways of constructing classes of structures from the bottom-up. Such methods have been based on electrostatics, shape and size complementarity, external stimuli (like magnetic field or light), and molecular and biomolecular interactions.[2−6] These methods all have pros and cons and work well for certain types of particles, in select environments, and in the context of particular uses. However, when it comes to employing chemical design to make targeted, highly ordered materials in an intuitive and deliberate way, colloidal crystal engineering with DNA offers significant advantages, not offered by other systems. It relies on the extraordinary chemical programmability of nucleic acids to direct the assembly of nanoparticles into highly ordered macroscopic architectures. Using principles that underpin this emerging field of chemistry, it has become apparent that we no longer must always play by nature's rules.[4,7]

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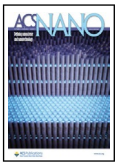

Table 1. Colloidal Crystal Engineering with DNA: Design Rules

	Design Rules
1	*The complementary contact model*: (a) (*solution*) PAEs will arrange themselves in a lattice (the most stable, thermodynamic product) that maximizes the number of all possible types of DNA sequence-specific hybridization (attractive) interactions and minimizes repulsive interactions. (b) (*crystal habit*) The thermodynamically favored crystal habit will be the one that exposes the closest-packed plane because it requires breaking the smallest number of particle-to-particle interactions per unit area and thus exposes the lowest-surface-energy facet. In the case of surface grown crystals, the orientations of crystalline thin-films will be dictated by the crystal planes that maximize complementary interactions with the substrate. Caveat: *Enthalpy vs entropy:* Long, flexible DNA linkers can be used to access entropy-driven crystallization regimes where unexpected phases, not predicted by the CCM for the given PAEs, can be attained.
2	*Hydrodynamic dimensions dictate assembly*: For spherical constructs, the overall hydrodynamic radius of a PAE, rather than the dimensions of the individual nanoparticles or oligonucleotide components, dictates its assembly and packing behavior.
3	*"Atom" (core particle) identity is separated from bonding characteristics (DNA recognition properties)*: Two particles with the same hydrodynamic ratio and DNA linker ratio form the same thermodynamic product, regardless of particle composition.
4	*Design by deletion*: The positions of the inorganic cores dictate the crystal symmetry of a lattice. Therefore, the use of a PAE with no inorganic core can be used to "delete" a particle at a specific site within a unit cell.
5	*Metallicity and particle-bond duality*: The size and DNA loading of a particle dictate its mobility in a lattice. Small particles, which have low numbers of DNA strands, do not occupy specific lattice sites in crystals of larger particles, but rather are mobile and often randomized.
6	*Complementarity*: (a) PAEs that have high size and shape complementarity produce higher quality crystals. (b) Shape complementarity and flexible DNA facilitate the formation of highly ordered space-filling structures (geometries that favor space tessellation) from one or more types of polyhedral space-filling or non-space-filling nanocrystals.
7	*Directional bonding and valency*: (a) Core shape can be used to introduce collective, directional interactions between building blocks. Anisotropic particles with flat faces, for example, will assemble into a lattice that maximizes the amount of parallel, face-to-face interactions between particles. (b) Directional bonding can also be achieved by creating anisotropic bond distributions on PAE surfaces or by using "molecular" PAEs with chemically well-defined topologies. (c) Hollow frame-like particles assemble via edge-bonding.
8	*Zone of anisotropy*: The zone of anisotropy is the phase space where the anisotropy of the particle is preserved in colloidal crystallization. The length of the DNA relative to the dimensions of the nanoparticle core (anisotropic) and its rigidity dictates the relative influence of these parameters. In general, longer oligonucleotides will minimize the influence of an anisotropic core.
9	*DNA bond versatility*: Crystallization depends on the nature of the DNA bonds. (a) DNA sequence and structure can be used to adjust bond length and strength. (b) DNA sequence can be used to control dynamic behavior; shorter, more AT-rich sequences will lead to weaker bonds and greater particle mobility. (c) DNA hairpins or DNA chemistry can be used to selectively address particular bonds in a lattice.
10	*Crystal growth and size*: Annealing at the melting (or bond dissociation) temperature of the DNA linkers will decrease crystalline defects and yield larger crystals.

In colloidal crystal engineering with DNA, the fundamental building blocks are nanoparticles (typically, with dimensions less than 100 nm) that are surface-functionalized with a dense layer of oriented nucleic acids (most commonly, single- or double-stranded DNA, 20−50mers). The prototypical nanoparticle building block is a spherical gold nanoparticle, functionalized with DNA in a dense radial orientation—a polyvalent DNA−gold nanoparticle (DNA-Au NP) conjugate, more recently termed a spherical nucleic acid (SNA).[2,8] Since the initial invention of this construct,[2] researchers around the globe have prepared hundreds of types from a variety of nanoparticle cores of differing sizes, shapes, and compositions and nucleic acid shells with differing lengths, densities, and flexibilities/rigidities.[9,10] These structures are often referred to as "programmable atom equivalents", or PAEs, and they have been cataloged conceptually in a nanoscale "Table of PAEs".[10,11]

Given the near-infinite number of PAEs that exist and the programmability of nucleic acids, colloidal crystal engineering with DNA has become one of the most versatile modes of crystal engineering of any type; it employs principles in chemistry combined with capabilities afforded by modern nanotechnology to address a materials chemistry grand challenge—creating programmable matter—oftentimes through inverse design. Over the past 30 years, DNA, in tandem with an extensive set of design rules (Table 1),[12] has become the source code for preparing thousands of structurally distinct colloidal crystals with over 90 different symmetries, including several that are not found in nature[12] and low-symmetry structures.[13,14] Single-crystal versions[15] can now be made, and in many cases, the properties of these crystals exceed those of their naturally occurring counterparts, especially in how they interact with light.[16−18]

Colloidal crystal engineering with DNA is also important because it offers a new way to think about chemistry and traditional concepts within it.[19,20] In the context of PAEs, the nanoparticle can be likened to the "atom", and the duplex DNA that connects the particles can be likened to a highly programmable "bond".[4] In other words, the nanoparticle is analogous to the atom's nucleus, and the single-stranded DNA recognition elements are analogous to the unpaired valence electrons that participate in bonding. However, unlike with atoms, colloidal crystal engineering with DNA hinges on a separation of the nanoparticle "atom" identity (a specific particle) from its bonding characteristics (DNA sequence) (Table 1, Rule 3). By drawing out this analogy, we have enriched and refined our understanding of the most fundamental aspects of matter and bonding as well as a wide range of other chemical concepts, spanning valency and Pauling's rules to valence shell electron pair repulsion (VSEPR) theory and phase diagrams.[1] Colloidal crystal engineering with DNA is not the only field where parallels to traditional chemistry can be drawn; for example, molecular machines and the mechanical bond,[21] supramolecular chemistry and coordination chemistry-based approaches to biomimicry,[22,23] and porous materials, such as metal−organic frameworks (MOFs),[24,25] offer rich material for this discussion. However, with colloidal crystal engineering with DNA, the analogy can be extended across many aspects of chemistry. Thus, colloidal crystal engineering with DNA offers, perhaps, the ideal system for making analogies to traditional chemistry, serving as a model for others to bridge traditional and modern scientific concepts and breathe new life into the field of chemistry, from the benchtop to the classroom.

In this Nano Focus, we provide a historical overview of colloidal crystal engineering with DNA. We start the discussion with the invention of the quintessential PAE, the SNA-gold nanoparticle conjugate, in 1996.[2] Then, we chart the trajectory of this field along parallel research tracks involving synthetic and other fundamental advances that laid the foundation for the emergence of PAE building blocks as powerful tools in materials science and medicine. We discuss the implications of

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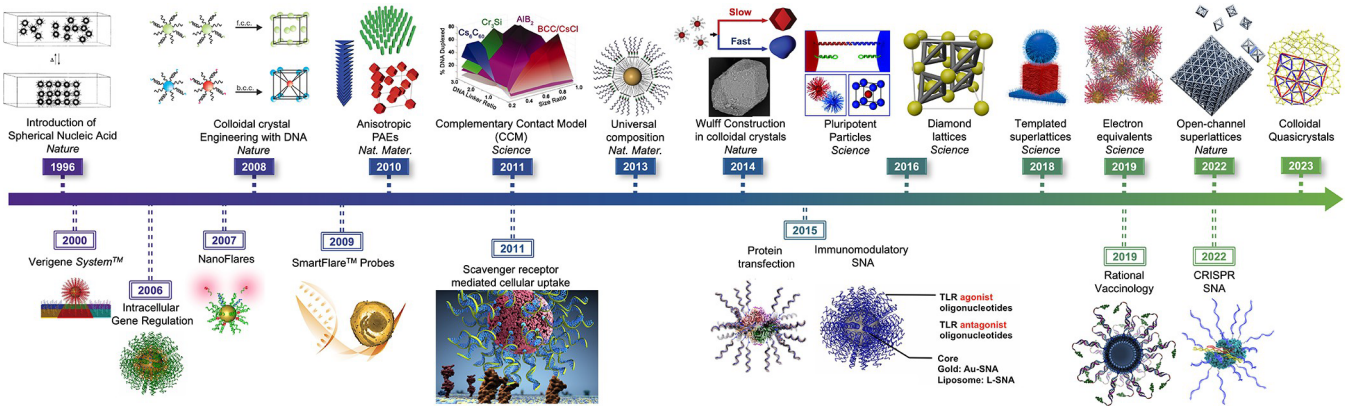

Figure 1. Timeline of selected key achievements pertaining to Spherical Nucleic Acids and Colloidal Crystal Engineering with DNA. The advances above and below the central arrow are more fundamental or more translational in nature, respectively. Above arrow, left−right: Material from ref 2. Copyright 1996, Springer Nature Limited. Material from ref 32. Copyright 2008, Springer Nature Limited. Material from ref 33. Copyright 2010, Springer Nature Limited. From ref 34. Reprinted with permission from AAAS. Material from ref 10. Copyright 2013, Springer Nature Limited. Material from ref 15. Copyright 2014, Springer Nature Limited. From ref 35. Reprinted with permission from AAAS. From ref 36. Reprinted with permission from AAAS. From ref 37. Reprinted with permission from AAAS. From ref 38. Reprinted with permission from AAAS. Material from ref 39. Copyright 2022, Springer Nature Limited. Image taken from material discussed at the Spring 2022 ACS National Meeting, San Diego. Below arrow, left−right: Denotes founding of Nanosphere in 2000. Image for 2000, 2006: Reprinted with permission from ref 8. Copyright 2012 American Chemical Society. From ref 40. Copyright 2014 National Academy of Sciences. Denotes founding of Aurasense in 2009. Taken from https://pdf.directindustry.com/pdf/merck-millipore/live-cell-rna-detection/31514-578889.html. Denotes founding of Exicure in 2011. Taken from https://www.youtube.com/watch?v=YxRQ1-MI24g. Reprinted with permission from ref 41. Copyright 2015 American Chemical Society. From ref 42. Copyright 2015 National Academy of Sciences. From ref 43. Copyright 2019 National Academy of Sciences. Reprinted with permission from ref 44. Copyright 2022 American Chemical Society.

colloidal crystal engineering with DNA in defining a new area of chemistry by drawing parallels to traditional chemistry, but perhaps more importantly, by highlighting the key differences that allow the modern researcher to move beyond it. Finally, we discuss how these building blocks and concepts allow one to make structures that are entirely new. We conclude with an outlook of the field, describing some of the challenges that remain and where we see this area of research heading in the short- and long-term. This Nano Focus is meant not only to inform the community about what has been done but also to impress upon it what may be possible in the future.

2. THE BIRTH AND GROWTH OF A NEW FIELD OF CHEMISTRY

2.1. 1996: The Spherical Nucleic Acid. The field of colloidal crystal engineering with DNA was born in 1996.[2] In a paper in *Nature*, we showed how one could prepare gold nanoparticles (~13 nm) surface-functionalized with alkylthiolated single-stranded DNA. Upon addition of a third DNA linking strand that was complementary to a portion of each of the DNA strands on the surface of the two sets of particles, and under the correct experimental conditions, these particles participated in hybridization interactions to form assemblies that were highly periodic (because particle−particle interactions were driven by DNA programmability) but not fully crystalline (because disordered aggregates rather than structures with long-range order were obtained). The formation of these assemblies from discrete particles in solution was accompanied by a red-to-blue color change due to the interactions of the localized surface plasmon resonances (LSPR) of the particles that were brought in close proximity through sequence-specific DNA binding events. Interestingly, the particle assembly event was reversible as a function of temperature (assemblies were observed at temperatures below the melting temperature of the hybridized DNA strands). This colorimetric change represented an amplification of the signal associated with hybridization/dehybridization that could be observed with the naked eye!

This initial 1996 study showed the power of using highly programmable chemical interactions to make materials and positioned DNA as a sequence-specific "glue" or bonding element in materials chemistry. Roughly a decade later, we would come to realize that this arrangement of DNA (or RNA) around a nanoscale spherical template defined an entire new form of nucleic acid, an SNA, with unique properties compared to nucleic acids of other shapes, like the linear and circular versions found in nature.[8] These conjugates laid the foundation for biological labels based upon well-defined nanocrystals modified with biorecognition elements,[26,27] including those made from quantum dots.[28] Indeed, in the decades that followed, many uses were found for the large class of biomolecule-functionalized metal, semiconductor quantum dot, and insulator particles, some of which became widely used in the burgeoning fields of biology and medicine.[29] For this reason, it is important not to confuse SNAs with constructs modified with a single strand of DNA[30] that are akin to a fluorophore-labeled oligonucleotide. Such structures do not impose directionality on the oligonucleotides, cannot be used to generate macroscopic crystalline materials, and are primarily useful for single-oligonucleotide labeling experiments. While these structures are important in their own right, especially as biological probes,[31] they are not SNAs and cannot be used for colloidal crystal engineering with DNA.

2.2. The Late 1990s to the Early 2000s: Structure−Function Relationships and the First Signs of Translational Significance. Based on the initial 1996 discovery, we, along with our collaborators, proceeded down multiple interconnected research tracks that have advanced through diverse phases of development (Figure 1). Momentum in one track has often fed evolution in the others, and foundational

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work in chemical synthesis and characterization have underpinned each scientific and technological advance.

SNAs and Their Properties. In any chemical system, what you cannot make and characterize, you cannot understand and apply. For this reason, in the late 1990s and early 2000s, our group and our collaborators like Robert Letsinger, SonBinh Nguyen, and George Schatz placed a heavy emphasis on refining synthetic techniques and expanding the types of nanoparticles and DNA that could be used to prepare nucleic acid−nanoparticle conjugates. For example, we incorporated other types of spherical metallic (e.g., Ag−Au core−shell)[45] and semiconductor (e.g., quantum dot)[46] particles as cores and explored the generality of the system by using nanoscale particles of different sizes[47] and DNA of different lengths and sequences.[48] We implemented different binding motifs beyond Watson−Crick pairing (e.g., G-quadruplex interactions[49]) and eventually synthesized asymmetrically functionalized nucleic acid−nanoparticle conjugates.[50,51] We also began to understand the implications of shape (the nanoparticle core being a template for a radial arrangement) and nanoparticle surface environment (a high density of DNA closely packed on a highly curved surface) on fundamental nucleic acid biochemistry, delineating key differences in binding behavior between the DNA-functionalized nanoparticles and linear forms of nucleic acids of the same sequence. In general, DNA-mediated nanoparticle assemblies have a higher melting temperature (an indicator of enhanced stability) and a sharper melting transition (an indicator of cooperative binding) than the same duplexed sequences free in solution.[48] Furthermore, DNA-functionalized nanoparticles bind complementary DNA strands more tightly (orders of magnitude) than a linear structure of the same sequence (an enthalpic effect resulting from DNA surface confinement).[52,53] The gold nanoparticle system also turned out to be ideal for elucidating distance-dependent optical properties for nanoparticles.[54]

Extracellular Biodiagnostics. Also, in the late 1990s and early 2000s and as a result of our initial observations pertaining to the chemical and physical properties of nucleic acid-functionalized nanoparticles, research in the Mirkin group focused heavily on exploiting them as probes in extracellular biodetection assays. In particular, the higher melting temperatures and narrower melting transitions for multivalent DNA-functionalized nanomaterials (as compared to the analogous linear strands), led to higher selectivities and sensitivities for assay targets and better mismatch discrimination compared to, for instance, DNA-functionalized fluorescent probes. This work ultimately popularized the concept of the "nanoparticle sandwich assay" for the detection of biomolecules, such as nucleic acids and proteins, and metal ions.[2,55−59]

The initial assays that employed these nucleic acid−nanoparticle conjugates involved colorimetric readout strategies, due to the optical properties of the gold nanoparticle core in the visible range of the electromagnetic spectrum. Over time, more complex extracellular nucleic acid-nanomaterial probe-based systems were designed based on electrical signal,[60] Raman signal,[61] light scattering,[62] and other forms of readout. Among the most impactful of these was the scanometric assay,[62] which was introduced in 2000. This assay forms the basis for the Verigene system, now sold by Luminex (a DiaSorin company) and used in many of the world's top hospitals. The bio-bar-code (BBC) assay,[55] introduced in 2003, is also important because it showed how nucleic acid nanomaterials and cleverly designed amplification steps, could be used to push the sensitivity and selectively of biodiagnostic tools beyond those conventionally used for nucleic acid and protein detection. The BBC assay redefined "undetectable" in the context of cancer biomarkers,[63] and we showed in several cases how it was extremely amenable to multiplexing.[64,65]

2.3. The Mid- to Late 2000s: Quantitatively Defining SNA Structure and Expanding the Scope of Structural Possibilities. Starting in the mid-2000s, we moved into a phase of exploration that drove major advances in materials chemistry and biomedicine. By adding a level of chemical precision to the system, we, for example, determined on average how many DNA strands were on the surface of the nanoparticles as a function of size and a variety of environmental conditions (e.g., [NaCl])[66] and further explored the role of surface curvature on nucleic acid loading.[67] We also prepared nucleic acid nanomaterials with different types of backbones, including those with densely packed and radially oriented LNA,[68] PNA,[69] and RNA[70,71] shells.

An Important Step toward Colloidal Crystal Engineering with DNA. With an enhanced understanding of the synthetic parameters and properties of SNA-gold nanoparticle conjugates, great strides followed in preparing more ordered and crystalline assemblies based upon them. In 2004, small-angle X-ray scattering (SAXS) was used to probe the structure of assemblies of nucleic acid-functionalized nanoparticles for the first time.[72] Although structure factors and nearest-neighbor distances were determined, evidence of only short-range, not long-range, order was revealed. Four years later though, in 2008, we, Schatz, and Byeongdu Lee, who would become a long-time collaborator,[32] and Oleg Gang's group[73] published back-to-back papers in *Nature* showing that nanoparticle crystallization could be precisely controlled using different DNA designs. Importantly, both groups of researchers realized the need to incorporate flexibility into the DNA strands to attain crystalline lattices with long-range order. Practically, the flexibility as well as the individually weak but collectively strong hybridization interactions between the particles (the weak polyvalent effect) allows reorganization to occur such that the system could attain the thermodynamically favored crystalline structure. We later showed how lattice parameters could be controlled with nanometer precision over the approximately 10 to 200 nm range by systematically changing the DNA length and nanoparticle diameter.[74] Indeed, we uncovered that there is a linear relationship between the gold nanoparticle nearest-neighbor distance and the total number of bases between gold nanoparticles; each base accounted for 2.6 Å in length on top of the combined dimensions of the gold nanoparticle radii and the two hexyl−thiol tethering moieties that also separated the gold nanoparticle centers. Based on this work, it quickly became apparent that this form of assembly offered a highly sophisticated route to the creation of vast new classes of programmable, designer matter.

Intracellular Diagnostics and Therapeutics. In the biomedical space, in 2006, we made the remarkable discovery that, when SNA-gold nanoparticle conjugates were introduced into cell culture (epithelial cells), the cells actively internalized them.[75] Through careful inhibition- and knockdown-studies, it was determined that the SNAs were taken up by scavenger receptor (class A)- and caveolin-mediated endocytosis in high quantities unlike their nanoparticle-free DNA counterparts.[76,77] Later, it was shown that this characteristic is general for SNAs, spanning over 60 different cell types, including stem cells. These nanostructures also resisted enzymatic degrada-

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tion[78] and did not elicit an adverse immune response.[79] Significantly, we now had a way to use SNAs to measure and manipulate *intracellular, not just extracellular*, contents.

This discovery led to the swift development of DNA-functionalized nanomaterials as gene regulation agents[70,75] and intracellular probes.[80] Specifically, DNA- and RNA-functionalized nanoparticles were found to be potent agents for gene regulation in antisense and RNAi pathways, respectively. These particles were designed to downregulate the expression of proteins associated with cancer in cells, tissues, animals, and ultimately humans. Because these particles were found to actively cross dermal, blood−brain, and blood−tumor barriers, skin[81] and brain cancers[82] were models for initial demonstrations. Teaming up with Amy Paller and Alex Stegh, we explored their potential in these arenas and later in the context of a variety of other cancers as well. The first-in-human clinical trials of nucleic acid-functionalized nanoparticles of this type, which targeted glioblastoma[83] and inflammatory markers in the skin associated with psoriasis, were run by Northwestern University and start-up companies spun out of it.

Moreover, DNA-functionalized particle-based intracellular detection of mRNA,[40,80,84,85] aptamers,[86] and other moieties in single, living cells was accomplished using "nanoflare" technology; a related system called the sticky-flare was developed for determining both the amount and spatial location of intracellular RNA.[87] Nanoflares were ultimately commercialized by AuraSense along with Merck/Millipore as Smart-Flares, and this platform was later enhanced through the development of FIT-flares.[88,89] In addition, we subsequently developed theranostic systems based on DNA-functionalized nanoparticles that could be used simultaneously for mRNA detection *and* gene regulation.[90] In systems of increasing sophistication, DNA-functionalized nanomaterials were also combined with chemotherapy (cis-platinum derivatives)[91] and imaging agents (MRI)[92] to perform multiple functions simultaneously.

2.4. The Late 2000s into the 2010s: PAEs, the Zone of Anisotropy, and the Rapid Expansion of Colloidal Crystal Engineering with DNA through the Development of Design Rules. *PAEs.* Until the late 2000s, our synthetic focus had historically centered more on making changes to the nucleic acid shell and much less on making changes to the nanoparticle core. In most studies, researchers relied on ~13-nm gold nanoparticle-based SNAs, due to a multitude of factors, including ease of synthesis, the stabilities of the gold particle and gold−thiol bond, and the vast body of knowledge that had been accumulated based upon this prototypical construct. During the next decade, we and others refined the ability to synthesize nanoconjugates of a variety of compositions and shapes and to stably modify them with nucleic acids, most commonly DNA, in such a way that their original structures and chemical and physical properties were retained. For instance, quantum dots[9] and spherical platinum,[9] silver,[93] iron oxide,[94] silica,[95] metal−organic framework (MOF),[96] liposomal,[97] polymeric,[98,99] infinite coordination polymer (ICP),[100] protein (including enzyme),[101,102] and micellar[103] nanoparticles were functionalized with DNA and found to exhibit many of the same hallmark properties as the gold-cored structures. DNA origami was also used as a fundamental building block.[104−106] In perhaps the most striking example, we found that even hollow spherical particles comprised of DNA cross-linked at one end also behaved like the core-filled structures from a biochemical standpoint.[107] Based on these results, the term "spherical nucleic acid" or "SNA" was introduced to describe what we had formerly been referring to as DNA-functionalized nanoparticles or polyvalent DNA-nanoparticle conjugates.[8] The descriptive naming was meant to capture the concept that these structures were new forms of nucleic acids with properties different from those of any other form of nucleic acid. The naming was important also to reinforce the idea that many of their properties are core-independent (a concept discussed in more detail below).

In the late 2000s and early 2010s, we also made substantial synthetic progress on the preparation of high-quality anisotropic particles and their functionalization with DNA for use in creating colloidal crystals.[33,108−111] We subjected these building blocks to many of the same rigorous structure−property−function analyses that we performed with the original spherical materials and were able to elucidate the fundamental implications of the shape of the particle template on the binding properties of the surface-bound DNA. As a result, bonding motifs came to light that were unique to systems comprised of anisotropic particles and that were observed because anisotropic particles, which enabled directional DNA interactions, mimicked the property of valency or coordination environment in traditional atomic systems (Table 1, Rule 7a). For example, we found that the use of DNA-functionalized triangular prisms in DNA-programmable assembly led to one-dimensional lamellar stacks of particles while octahedra and rhombic dodecahedra assembled into orientationally ordered body-centered cubic (BCC) and face-centered cubic (FCC) phases, respectively.[33] Given that the majority of the DNA on these structures resides on the flat faces, not the edges, these arrangements maximized DNA bonding and therefore were thermodynamically favored, in line with what would come to be known as the complementary contact model (CCM, *vide infra*).[34] We, in collaboration with Monica Olvera de la Cruz's group, also identified "zones of anisotropy" for particles of different shapes by investigating the effects of particle core (via the particle symmetry and particle size) and ligands (via the ligand length) on crystallization.[112] The zone of anisotropy is the portion of the phase space where the anisotropy of the particle core is retained and imposes directionality on the DNA (Table 1, Rule 8).

Moreover, this library of isotropic and anisotropic materials and the introduction of general strategies for the functionalization of any type of material with DNA led to the idea of the "programmable atom equivalent" or "PAE" in 2013.[10,11] PAEs are any of the variety of nucleic acid-functionalized nanoparticles of different sizes, shapes, and compositions, of which SNAs are one class. This library of structures and conceptual framework led to our delineation of a nanoscale "Table of PAEs," which offers a way to think about cataloging PAEs based on structure. Unlike the Periodic Table of Elements, the nanoscale Table of PAEs is not periodic but near-infinite in nature because of the multitude of sizes, shapes, and compositions of PAEs that can be prepared.

Design Rules for Colloidal Crystal Engineering with DNA. We, along with Lee and Schatz, published what became a landmark paper in *Science* in 2011 that delineated the first set of design rules for synthesizing colloidal crystals of a variety of different symmetries, including FCC and BCC, but also CsCl, AlB_2, Cs_6C_{60}, hexagonal close packed (HCP), and others.[34] In this study, we introduced and explained the CCM (Table 1, Rule 1a). The CCM states that PAEs seek to maximize DNA hybridization (or bond formation) to form the thermodynami-

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cally most favorable crystalline structures via physical contact and duplex formation. In other words, one can design structures based upon intuitive geometric arguments that arrange the particles in a given system into a configuration that maximizes bonding. This study enabled the formulation of a phase diagram that spotlighted the immense predictive power of the design rules (Figure 1). Indeed, these rules were even later shown to be applicable to significantly larger (micrometer-sized) particles assembled using DNA by David Pine and Marcus Weck.[113] Shortly after our initial 2011 study, hollow, coreless spacer PAEs (that were X-ray transparent) were used to occupy lattice sites normally occupied by inorganic particles.[12] When the design rules were applied, conventional nanoparticle lattices (e.g., graphite, AB_2) as well as a non-natural lattice ("lattice X") using the concept of "design by deletion" were prepared (Table 1, Rule 4). Methods were also subsequently developed to stabilize colloidal crystals, for example, by encasing them in silica, so that they could be more readily characterized and so that their overall applicability could be increased.[114−117]

Around the same time, a set of related design rules were being developed to create highly crystalline, ordered lattices on surfaces (two-dimensional versions of colloidal crystals akin to those that were being prepared in solution). For example, in the context of DNA-programmable superlattices grown on substrates, we found that nanoparticle superlattices will adopt an orientation that maximizes complementary DNA interactions with a given crystal plane[118] (Table 1, Rule 1b); this rule can be viewed as a version of the CCM that applies when a substrate is integrated into a system of PAEs. Accordingly, we worked with Harry Atwater and Robert Macfarlane and found that one can use the appropriate surface modification methods along with high-resolution patterning capabilities to exquisitely control crystal growth and orientation in a pseudoepitaxial manner.[119] We, in collaboration with Macfarlane, also demonstrated heteroepitaxy and elucidated strain dissipation mechanisms by exploiting DNA design.[120] Macfarlane later showed control over crystal texture (the relative orientation of crystal grains in a polycrystalline material) as well.[121] In general, as with the nonsurface confined systems, where, with atoms, epitaxial growth is constrained by nature, PAE and substrate design can be independently tuned to control the crystallization outcome on surfaces. Importantly, substrate-based, two-dimensional PAE assembly, like solution-based, three-dimensional assembly, highlighted that DNA flexibility and the weak polyvalent effect was critical in forming highly crystalline structures.[122]

From there, new design rules (Table 1) were added to the list to create colloidal crystals with increasing complexity, including ternary colloidal crystals via topotactic interconversion strategies,[123] cocrystals of multiple types of anisotropic particles,[124] lattices based on DNA and/or RNA bonds,[125,126] and protein-based crystalline lattices,[101] based on nucleic acid programmability. In 2016, the Gang group developed a diamond family of superlattices,[36] and in 2017, we synthesized anisotropic bipyramidal gold-based PAEs and, in collaboration with Glotzer, studied their assembly to make clathrate colloidal crystals.[13] Lattices with this structure had previously been inaccessible with isotropic particles and, to this day, represent some of the most complex architectures (based upon unit cell size) made using any form of colloidal crystal synthesis or engineering method. In addition, colloidal crystals were prepared where lattice parameters and/or symmetry could be toggled using external stimuli like pH,[127] light,[128] or magnetic fields,[129,130] experimental parameters like solvent,[131] hydration state,[132] cation type,[133] and DNA strand displacement-based designs (Table 1, Rule 9).[134] Systems that employ DNA hairpins are particularly interesting because PAE building blocks could be driven to different crystalline end points using the same PAE starting materials where different surface-bound hairpins were addressed separately with single-stranded chemical cues. In this way, the PAE building blocks, using the atom analogy, were transmutable.[35] Also, during this time, the importance of the DNA bond was further investigated as well as the implications of the competition between enthalpic and entropic forces and attractive and repulsive interactions on colloidal crystallization (Table 1, Rule 1 caveat).[135−138]

From Polycrystalline Materials to Single Crystals. A few years after the publication of the initial design rules for colloidal crystal engineering with DNA,[34] we began to explore and understand the implications of the CCM on our ability to control colloidal crystal habit, or its overall external shape. In a paper published in 2014, we and Olvera de la Cruz showed how spherical PAEs could be used to form single crystals with BCC or CsCl symmetries and a rhombic dodecahedron shape, the Wulff shape for this system.[15] The rhombic dodecahedron habit, in this case, is thermodynamically favored because it exposes the closest-packed plane, breaking the smallest number of PAE interactions per unit area and exposing the lowest-surface energy facets (Table 1, Rule 1b). This tenet can be used to predict crystal habit based on PAE structure and the CCM. For example, a cubic habit was attained from cube-shaped PAEs, a rhombic dodecahedron habit was formed using truncated octahedron-shaped PAEs, and an octahedron habit was obtained with rhombic dodecahedron-shaped nanoparticles.[139] Methodologies for growing nonequilibrium crystal shapes (those with anisotropic habits) were also introduced.[140] In addition, surfaces later were used to template colloidal crystals with desired habits with position and orientation control.[141,142] For example, Macfarlane and his team showed that the surface-bound Wulff polyhedra (called Winterbottom constructions) could be synthesized by assembling PAEs on DNA-coated substrates. In such structures, the CCM can be applied to tailor the crystallite shape by altering its orientation or the relative strength of the PAE−PAE and PAE-substrate interactions.[143] Surfaces were also ultimately used with anisotropic shapes to access low-density 2D lattices not obtained with conventional entropy-driven crystallization.[144]

Tailorable Single Crystalline Materials and Their Impact on Catalysis and Optics. The significant synthetic advances and fundamental understanding about binding garnered in the previous 10 years, came to a head in the mid-2010s. Around this time, major advances in colloidal crystal engineering with DNA as it applies to catalysis, photonics and optics, and biomedicine (especially in immune-engineering) were made. In 2015, we synthesized the first catalytically active particle superlattices assembled using DNA interactions.[145] Here, the DNA-assembled gold nanoparticle superlattices encased in silica were shown to be catalytically *inactive*, but upon calcination to remove the DNA, they were catalytically active with respect to alcohol oxidization.

Through collaborative work with Schatz and later with Koray Aydin, we showed how one could tune the interactions between light and dye-free and dye-functionalized gold nanoparticle-based single colloidal crystals using colloidal crystal engineering with DNA.[16,146,147] We also identified a

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polarization dependence in single crystals of spherical PAEs and anisotropic octahedral particles, a consequence of particle shape and the positional and rotational order of the particles in the assembly.[148] This line of inquiry led to the derivation of a set of design principles for making photonic crystals with desired photonic stopband properties by taking advantage of DNA spacer groups, leading to the formation of highly reflective structures.[149]

Similar studies were conducted on surfaces as well, and in 2018, we, along with Adyin's and Vinayak Dravid's groups, used pore-patterned surfaces and DNA programmable assembly to study and control light−matter interactions. Through this work, a broadband absorber with a solvent polarity response was identified.[37] Later, device-quality optical metamaterials were prepared as well as optical metacrystals with unnaturally high refractive indices and low losses that gave rise to multipolar Mie resonances.[17] We and Schatz delineated an experimental and theoretical framework for how crystal habit design can modulate far-field extinction and light confinement in plasmonic metamaterial superlattices.[150] Macfarlane also demonstrated that plasmon-based heating with PAEs enables localized crystallization and assembly,[151] and his group also showed that the ability to deterministically produce single crystal PAE lattices of defined shapes enables their use as components of optical devices, specifically micromirrors.[152] Systems that feature non-natural optical properties are discussed more below.

SNA-Based Immunotherapies and Tools for Gene Editing. By the mid-2010s, roughly 10 years had passed since SNAs were first put into cells. Because the efforts in using SNAs as intracellular probes and gene regulation agents had progressed to more translational pursuits, we shifted the focus of our fundamental work toward SNA-based immune-engineering. In 2015, we, working closely with a team at Aurasense Therapeutics, made the first observations that SNAs prepared from immunostimulatory CpG DNA could be used to elicit a potent innate immune response.[42] We also identified another important fundamental biochemical property of these SNAs that, when prepared with CpG DNA, they engage with toll-like receptors (TLRs), the structures that control immunity in living systems, in previously unrecognized ways. Specifically, CpG SNAs bind TLRs orders of magnitude more tightly than free CpG DNA of the same sequence.

By taking advantage of SNA modularity and integrating antigens (as well as the nucleic acid adjuvants) into the SNA design, in 2019, we explored this system in the context of adaptive immunity, introducing the concept of "rational vaccinology" with Bin Zhang's group.[43,153] The concept was established based upon the hypothesis that the structure, not simply the composition, of a vaccine is critical in dictating the most potent immune response; this seemed intuitive since structure can control signaling kinetics and potency. Through SNAs and this approach, we have shown that targets that had previously failed out of clinical trials could gain potency when arranged in a properly structured SNA formulation.[154] Furthermore, multiple antigens have been incorporated in SNA vaccines,[155] and the importance of antigen release kinetics on potency has been determined.[156] Given the thousands of possible SNA (and PAE) formulations, combinatorial and machine learning approaches have been brought to bear on SNA therapeutic design to find the most potent structures with Andrew Lee, Neda Bagheri, and Milan Mrksich and their teams.[157]

Follow-up studies based upon the SNA platform have shown the power of rational vaccinology in constructing potent vaccines across nine different cancer models and a model for infectious disease (i.e., COVID-19).[158,159] Significantly, the principles of rational vaccinology and structural immunotherapy, more generally, have been delineated, not just on the benchtop, but in the clinic in patients, with, for example Merkel cell carcinoma (MCC) and cutaneous squamous cell carcinoma (CSCC), deadly forms of skin cancer. An SNA immunotherapy based on TLR9 agonists was shown to be superior to linear TLR9 agonists both in terms of its cellular uptake and TLR9 activation. These features resulted in superior *in vivo* efficacy, tumor shrinkage in multiple tumor types, and durable long-term immunity in mice that were dosed with the SNA drug in combination with a checkpoint inhibitor therapy. And, strikingly, this SNA drug cured late-stage, metastatic MCC in three patients, including a 92-year-old male with metastatic MCC, in Phase 1b/Phase 2 human clinical trials (NCT03684785). These patients were shown to be refractory to checkpoint inhibitor therapy (Pembrolizumab) and had failed all other therapies. This example represents perhaps the first clinical evidence of the importance of "structural immunotherapy." In a related area, intracellular "nanoflare"-inspired, quencher-free detection schemes were developed based on forced intercalation and protein-based structures with increased applicability and decreased background signal and false-positives.[160] Moreover, protein-core SNAs were utilized in therapeutic protein delivery[41,161] and to generate CRISPR SNAs[44] aimed at expanding cell and tissue access for this important gene editing platform.

2.5. The Late 2010s to the Present Day: Molecular SNAs, Electron Equivalents, and Hollow Frame PAEs. *Molecularly Precise Building Blocks.* Returning to synthetic and fundamental advances, over time we began to recognize that our inability to precisely know the chemical composition and properties of every individual PAE in a given sample (versus the average of the structures and properties in a bulk sample of millions or billions of PAEs) presented challenges. In other words, we realized that we did not have a clear path to understanding the structure and properties of PAEs on a molecular level like one could with small molecules using conventional chemical analytical techniques (i.e., NMR). This inability became an issue because many materials science and biomedical applications of PAEs rely heavily on PAE uniformity.[162] Thus, in 2018, "molecular" versions of SNAs, where the chemical identity and structure of the core and the shell could be precisely known, were created.[163] It was important to ensure, however, in such systems that the structural features of SNAs that give rise to their unique functional properties were maintained.

Thus far, several types of "molecular" SNAs have been introduced, including ones with molecular T8 polyoctahedral silsesquioxane and buckminsterfullerene C_{60} cores, where, by having a set number of chemical binding locations for the DNA strands in the shell, the exact DNA loading of each structure can controlled.[163] Systems were also developed where dibenzocyclooctyne (DBCO) moieties with variable valency and dodecaborane clusters with pentafluoroaryl-terminated linkers (that could be coupled with thiolated molecules and macromolecules) were used as cores.[164−166] The DBCO cores were functionalized with DNA or DNA dendrons, which can be thought of as a wedge-shaped portion of a three-dimensional SNA. Moreover, we prepared smaller,

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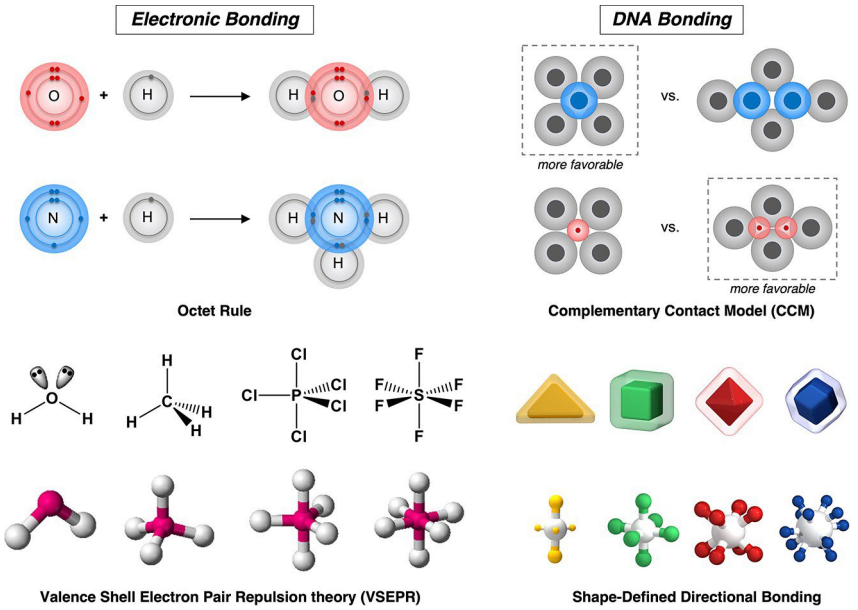

Figure 2. Comparison of electronic bonding in conventional chemistry (left) with DNA bonding in colloidal crystal engineering with DNA (right), which represents a type of chemistry at the nanoscale. Top right (Octet Rule): Note that both inner and valence shell electrons are shown. Bottom right (Shape-Defined Directional Bonding): Reprinted with permission from ref 112. Note that the smaller yellow balls in the bottom left image in this quadrant denote that bonding also occurs on the edges of a prism albeit to a lesser extent than on the faces. Copyright 2016 National Academy of Sciences.

cluster-like PAEs (particles that are less than 1.5 nm in diameter and that have countable numbers of DNA strands appended to them)[38] and SNAs with 1.4 nm Au_{102} nanocluster cores.[167] Routes to corner-, edge-, and facet-controlled growth on nanocrystals were also revealed[168] as well as porous PAEs,[39] including triangular, cubic, and octahedral nanoframes, and we developed methods for generating colloidal crystals that were larger (up to 21 μm)[169] and even macroscopic in size (greater than 100 μm).[170]

Electron Equivalents in Colloidal Crystal Engineering. These unusual materials presented expanded possibilities for exploring and utilizing PAEs in colloidal crystal engineering with DNA. In 2019, using what we call "electron equivalents" (EEs) as binding elements, we, Olvera de la Cruz, Dravid, and Lee discovered and described a property of colloidal crystals, termed metallicity.[38] These systems were comprised of large 10-nm gold PAEs with a dense coating of DNA and small 1.4-nm gold EEs grafted with a low number of DNA strands (less than 6). In this system, the EEs are complementary to the PAEs, and the PAEs cannot form crystals on their own. We found that the EEs do not occupy specific lattice sites but rather diffuse through the lattice in a manner reminiscent of classical electrons in metals (described by the Drude model[171,172]) (Table 1, Rule 5). New phases[38] could be made by tuning the EE:PAE ratio in solution and the total DNA coverage on the EEs. EE delocalization and diffusion were explored by tuning EE bonding strength and temperature to gain a better understanding of this unique property. EEs were ultimately used to make types of colloidal crystal "alloys": interstitial, substitutional, phase-separated, and intermetallic.[173] They were also used to break symmetry in isotropic systems of PAEs by creating anisotropic distributions of "valence" EEs around PAEs.[14] Likewise, "molecular" EEs with DBCO cores were prepared and used in colloidal crystal engineering with DNA,[165] and DNA dendrimers with DBCO cores were integrated into colloidal lattices, enabling, for instance, the formation of a low-symmetry Ti_5Ga_4-type phase that had yet to be reported in the field.[164]

Hollow Frame PAEs and Porous Colloidal Crystals. From a more applied standpoint, the advent of reliable routes to hollow frame materials paved the way for the formation of open-channel colloidal crystals via edge-bonding (Table 1, Rule 7c).[39] These structures are interesting fundamentally, but also offer the ability to perform host−guest-type encapsulation processes in their channels. Indeed, they provide access to pore dimensions over the 10−1,000 nm length scale, larger than those of metal−organic frameworks (MOFs) but smaller than those of lattices prepared using additive manufacturing. Their structures are reminiscent of top-down fabricated split-ring resonators and are excellent candidates for use as optical metamaterials, including those that exhibit negative refractive indices (NRI). When an ultrathin monolayer of these porous PAEs were prepared on surfaces, broadband metasurface absorbers, whose synthesis did not rely on cumbersome lithographic steps, were reported.[174]

Also, once we were able to prepare larger (macroscale) colloidal single crystals, we with Lee, Aydin, and Glotzer were able to substantively explore their mechanical properties. In 2022, we determined that these larger crystals have high viscoelastic volume fractions (more than 97%).[170] As a result, after deformation, they quickly reassume their initial well-formed crystalline morphology and internal nanoscale order. It is interesting to note that, for most crystals, such compression and deformation would have been permanent and irreversible. The observed structural changes were accompanied by reversible optical property changes. Before and after deformation, the crystals exhibited near-perfect (over 98%) broadband absorption in the ultraviolet−visible region, but the deformed crystals exhibit significantly increased reflection (up to 50% of incident light at certain wavelengths).

"Molecular" SNAs in Biomedicine. The development of new SNA moieties also opened up opportunities within

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biology and medicine. We discovered that entities, such as small molecules, DNA/RNA, peptides, and small proteins, could be conjugated to DNA dendrons and facilitate their cellular uptake (for example, >20-fold increase in DNA delivery per cell compared with their linear counterparts).[175] These structures showed no apparent toxicity and, because one could precisely control the number of branches on each DNA dendron, we were able to directly relate increasing DNA density to higher levels of uptake. Later, we demonstrated the power of DNA dendrons as molecular vaccines in cancer models *in vivo* and leveraged their molecular precision to buttress the case for rational vaccinology − the idea that vaccine structure can be as important as vaccine components in determining efficacy.[176] These molecular materials have formed the basis for Flashpoint Therapeutics, which is commercializing them in the context of cancer immunotherapy.

As we look forward to what's next, it is important to look back and take stock of where we have been and how we got to where we are today. The story of colloidal crystal engineering with DNA using SNAs, or PAEs, more broadly, is one that shows how fundamental discoveries across seemingly disparate fields of basic science and engineering can rapidly lead to translated tools and technologies with significant societal benefit.

3. A FORM OF CHEMISTRY AT THE NANOSCALE

3.1. The Nanoparticle "Atom" and the DNA "Bond". Over the past three decades, we have analyzed the similarities and differences between conventional chemistry concepts pertaining to atoms and bonds and a new chemistry at the nanoscale involving nanoparticle "atoms" and DNA "bonds".[4,19,20] As mentioned above, this concept is underpinned by the analogy of the nanoparticle being the nucleus of the atom, and the DNA being the unpaired valence electrons that comprise the bonding elements. Under the right experimental conditions and using chemical design, we can program the interactions between PAEs to create extended assemblies in ways not possible with traditional atoms, where we do not possess the same control over the fundamental building blocks and their properties.

3.2. The Complementary Contact Model and the Octet Rule. The library of PAEs that has been developed presents an unprecedented opportunity to challenge how we think about bonding, in general. In fact, the Complementary Contact Model (CCM) in PAE bonding is analogous to the Octet rule in conventional chemistry. The Octet rule[1,177] is a relatively simple model used to predict and describe atomic bonding through valence shell electron interactions. According to this rule, atoms have the most stable configuration when they have a full shell of eight valence electrons and maximize their bonding until they reach that arrangement (Figure 2). Likewise, according to the CCM, the thermodynamically most favorable crystal structure is the one that maximizes DNA hybridization interactions between PAEs.

3.3. Bond Order, Length, and Strength. In natural systems, bond order is the number of bonding pairs of valence electrons between two atoms.[1] Bond order is dictated by the atoms involved in a given bond, and it is correlated with bond strength and bond length. For instance, multiple bonds are possible when multiple pairs of electrons are shared between nuclei, and these bonds (i.e., double, triple) are progressively stronger and shorter than single bonds. However, unlike natural systems, "bond order" in DNA programmable assembly is not dictated by nanoparticle atom identify, and it is not always correlated with bond strength and length. In one example, branched bonding motifs have been used, with doubler and trebler multivalent organic moieties, leading to increased "bond order" and thermal stability as well as expanded structural possibilities.[178] In another example, DNA intercalators have also been used to enhance bond strength in colloidal crystals.[179] Interestingly, in this example, bond order is decoupled from bond strength. For the same number of DNA linkages ("bond order"), bond strength was modulated by changing the chemistry and concentration of the ruthenium-based DNA intercalator used. It was also found that, in this particular case, a longer bond was correlated with an increased bond strength, the opposite of what is seen with atomic systems. This difference calls attention to the fact that, while analogies to general chemistry are conceptually useful, they are simply a model to compare, contrast, and better catalog the behavior of these colloidal systems.

3.4. Bonding and Metallicity. In general, PAE bonding to form colloidal crystals can primarily be thought of as "ionic" in nature. PAEs can be programmed to form crystalline lattices in a manner similar to how Na^+ and Cl^- come together to form the structure described by table salt (NaCl), for example, or how other atoms come together to form a multitude of other symmetries. Because of the nature of the mobility of electron equivalents (EEs) in lattices of PAEs, we can describe bonding in these systems as more "metallic" in nature.[38] At lower temperatures and higher EE DNA loading, the EEs are fixed in clusters relative to the PAEs and their diffusion is minimized or prevented. However, at high temperatures and low EE DNA loading, the EEs diffuse within the lattice of PAEs reminiscent of how electrons behave in the electron-sea model describing metallic solids.[1] Interestingly, note that the conductivity of metals actually decreases at higher temperatures, the opposite of what is seen with EEs and PAEs. Again, one can see that although analogies to general chemistry are conceptually useful, one must remember that the underlying chemistry of atomic and PAE systems are fundamentally different.

3.5. Symmetry-Breaking and VSEPR Theory. Colloidal crystal engineering with DNA also offers an avenue for a rich analogy to molecular geometry and the VSEPR model (Figure 2). Molecular shape in three-dimensions is a result of the directional overlap of orbitals of electron density around central atoms, and VSEPR theory allows the chemist to predict molecular shape. Again, nature ultimately dictates the basic features of atomic orbitals, and the chemist can do little more than understand and catalog nature's design rules. With colloidal crystal engineering with DNA, we have discovered multiple ways to break symmetry in bonding interactions.[180] For instance, we have shown that the shape of the nanoparticle can be used to dictate valency and coordination geometry; anisotropic nanoparticle templates, like cubes, triangular prisms, or octahedra, impart bond directionality by orienting the bonding elements (the DNA strands) in space.[33] Such directionality has also been achieved by chemically controlling the location of bonding elements on otherwise isotropic PAEs;[50,51] this can be done using proteins with surface amines and thiols that have orthogonal chemical reactivities that can be selectively accessed for DNA functionalization (Table 1, Rule 7b).[181,182] In addition, anisotropic distributions of EEs in lattices of PAEs have also been used to break symmetry and impart directional bonding possibilities; in particular, systems

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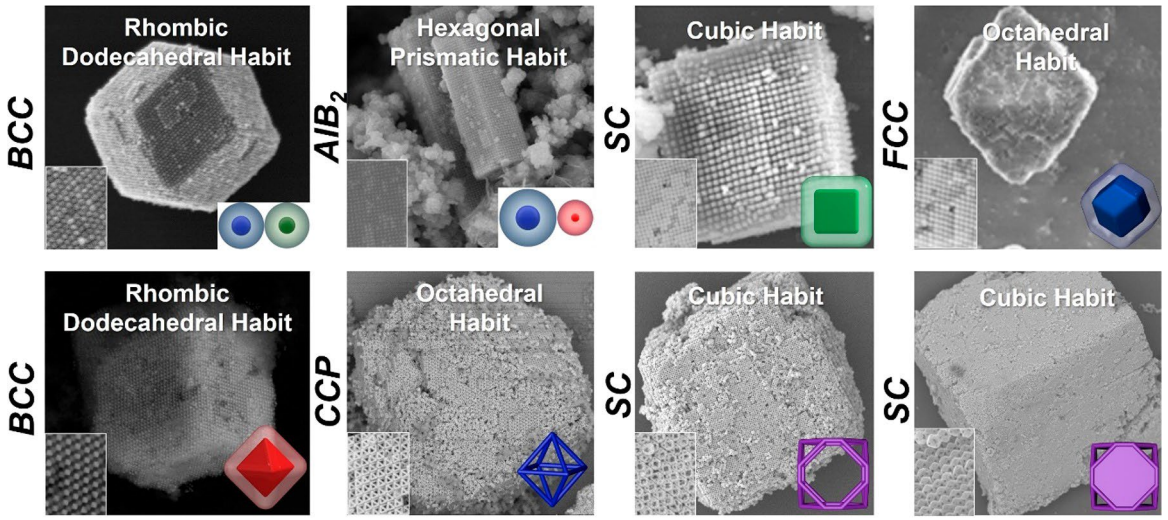

Figure 3. Examples of colloidal crystals that can be prepared using colloidal crystal engineering with DNA. Top, left−right: Material taken from ref 115. Copyright 2018 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. Material taken from ref 140. Copyright 2018 Springer Nature Limited. SC and FCC: reprinted with permission from ref 139. Copyright 2016 American Chemical Society. Bottom, left−right: Image based on work in ref 148. CCP, SC, and SC. Material taken from ref 39. Copyright 2022 Springer Nature Limited.

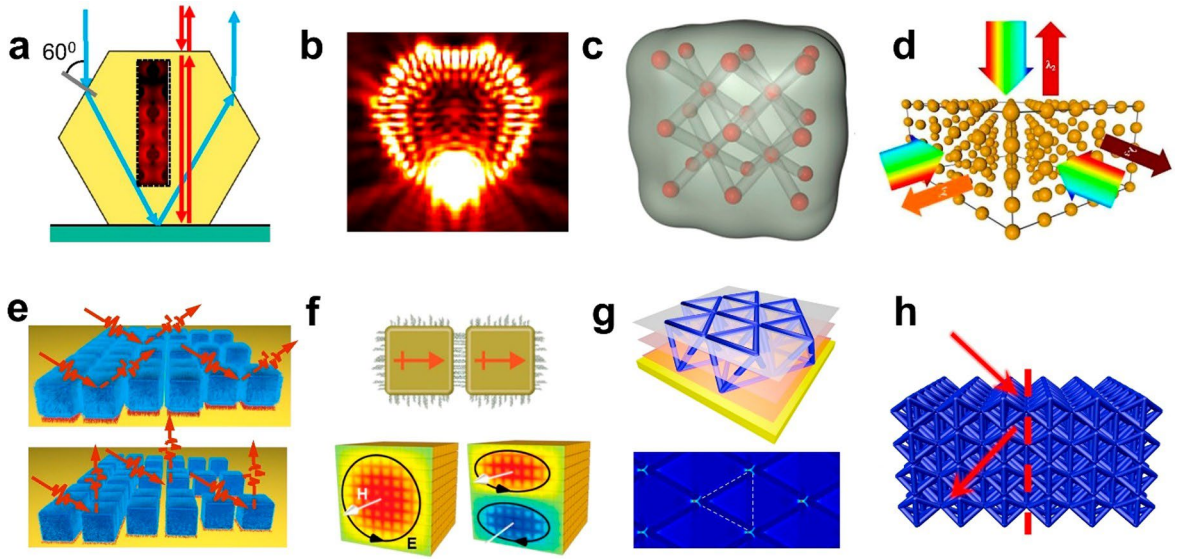

Figure 4. Colloidal crystals, engineered using DNA, display many interesting optical and catalytic properties. (a) From ref 16. Copyright 2015 National Academy of Sciences. (b) From ref 146. Copyright 2015 National Academy of Sciences. (c) Reprinted with permission from ref 145. Copyright 2015 American Chemical Society. (d) Image associated with work in ref 149. (e) From ref 18. Copyright 2020 National Academy of Sciences. (f) Reprinted with permission from ref 17. Copyright 2020 American Chemical Society. (g, h) Material taken from ref 174. Copyright 2022 WILEY-VCH Verlag GmbH & Co. KgaA, Weinheim.

with mobile, delocalized EEs lack valency, and those with fixed, localized EEs contain aspects of valency.[14] Also, symmetry-breaking has been accomplished with DNA dendrimer synthons (Table 1, Rule 7b).[180]

3.6. The PAE Design Rules and Pauling's Rules. Moving forward, it is likely that colloidal crystal engineering with DNA and PAEs will evoke other types of behavior commonly seen with atoms and molecules, adding to the framework of analogies presented here. Such analogies are excellent for guiding our thinking about the connections between traditional and modern chemistry. But, an arguably more significant take-home message is that, the chemistry is fundamentally new in many ways. Unlike traditional chemistry, bonding with PAEs and EEs is core- (or nanoparticle "atom"-) independent; the design rules for colloidal crystal engineering with DNA apply for all of the PAEs in our vast library, giving the chemist great control. This control allows for the realization of interesting multicomponent systems, accessible via phase diagrams (much like those used in conventional chemistry or materials science to describe phase behavior or metal alloy miscibility).[34] In the PAE case, we have identified that the PAE size ratio and DNA linker ratio (not the core composition or absolute size) dictates the thermodynamically favored crystal structure because these parameters dictate crystal stability, and we have constructed phase diagrams relating DNA linker ratio and size ratio.[7,34] These phase diagrams have been used to understand and predict which structures will form from particular PAE starting materials.[7,34]

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As such, the design rules (and the phase diagrams that can be built based on them) offer tremendous predictive power. In this way, the design rules for colloidal crystal engineering with DNA are more versatile than Pauling's rules for inorganic solids, which allow one to rationalize why certain inorganic structures form, as opposed to using them to design and realize target architectures.

4. CHEMISTRY THAT INSPIRES BEYOND NATURE

Using the design rules and phase diagrams as well as advances in nanosynthesis and characterization, we have realized materials (Figure 3) with a variety of interesting and useful properties. Because metallic nanoparticles (a primary type of building block used in colloidal crystal engineering with DNA) have interesting optical properties (the individual particles as well as the collections of them in close proximity), optics was one of the first areas of science upon which colloidal crystals have been brought to bear (Figure 4).[16,183]

Optical metamaterials, which can be used to manipulate light in ways not typically seen with ordinary materials, have been a popular synthetic target in the context of engineered matter.[184] Metamaterials, of which an invisibility cloak is an oft-cited example, are materials with properties that do not exist in nature; for instance, they can display non-natural optical responses, like near-zero or negative refractive index (NRI). Optical metamaterials, including those based on nanoparticles and DNA programmable assembly, are attractive for a variety of applications, including those that involve dynamic lasers and lenses, imaging, computing, and communications.[185,186]

Colloidal crystal engineering with DNA has been used to construct classes of dynamic three-dimensional metacrystals as well as metasurfaces, their two-dimensional counterparts. In one demonstration, single-crystalline colloidal metacrystals were constructed from gold nanocubes using programmable DNA interactions.[17] We, Schatz, and Aydin found that these metacrystals exhibit unnaturally high refractive indices that give rise to mid-infrared Mie resonances in crystals that are tunable based upon size. We, Aydin, and Glotzer have also shown versions of colloidal crystals to be near-perfect (over 98%) in terms of their broadband absorption in the UV region.[170] In the context of two-dimensional metasurfaces, substrates, including templated ones,[187] PAEs and/or the design rules for their assembly were utilized to experimentally realize theoretically predicted particle arrays that behave as reconfigurable anomalous reflectors,[18] epsilon-near-zero (ENZ) materials,[188,189] and high (~90%), and polarization-independent absorbers in the visible region.[174] Much work remains to be done in this area, and our lab is continuing to develop methods for attaining materials with unusually low symmetries,[183] for example, space-filled (tessellated) and non-space-filled architectures, like quasicrystals. Such materials will likely display an interesting host of nonlinear optical responses, like second-harmonic generation (SHG), as well as birefringence and dichroism. Non-DNA based assembly methods for producing low-symmetry metamaterials, like those from Matthew Jones and his group, highlight the appeal of scalable methods for the formation of advanced optical structures.[190]

In addition, recent synthetic advances, including those geared toward attaining uniform[191,192] and large (greater than 100 μm in size) colloidal single-crystals, have opened up a route for us and others to probe their mechanical properties.[170,193] Interestingly, hyperelasticity was observed for rhombic dodecahedra comprised of spherical gold nanoparticle crystals.[170] Upon dehydration, the crystals were compressed into irregular shapes, and, upon rehydration, they assumed their initial morphology and internal structure within seconds. This shape memory was a consequence of the flexible and dynamic nature of the DNA bonding that exists between PAEs and is not typically seen with other types of crystals. In other words, for most crystals, such compression and deformation would lead to permanent, irreversible damage. The Macfarlane group also demonstrated that, by controlling PAE design, one could enable the synthesis of materials with independent control over modulus and yield strength, two mechanical parameters that are typically linked in bulk materials.[193] Moving forward, porous, anisotropic PAEs, offer one possibility for making assemblies with tunable stiffness and strength. These architectures,[39] which have been shown to host complementary DNA-modified gold nanoparticle guests, also provide an interesting route to mechanical metamaterials as well as interesting catalytic structures. Importantly, advances in synthesis and characterization capabilities, including ones that are becoming so sophisticated that we can watch nanoparticle growth and assembly occur in real-time,[190,194,195] will allow us to redefine what's possible in optics, catalysis, mechanics, and medicine, and the desire to surpass what is possible in nature in these areas will drive researchers to make and understand ever more complex nanostructures.

5. FINAL THOUGHTS AND A CALL TO ACTION

As chemists, we are living in exciting times. In the case of colloidal crystal engineering with DNA, it is remarkable to realize how far the field has come over a relatively short period of time; it has evolved in ways and led to discoveries and innovations in chemistry that nobody could have predicted or anticipated in 1996. Over the past 30 years, from the birth of what is now called the SNA to the synthesis and characterization of hundreds of different versions of them, from their development as chemically well-defined diagnostic probes to their use as gene regulation/editing and immunotherapy agents to manipulate the biological contents of cells directly to combat disease, from the rudimentary design of amorphous aggregates to the creation of colloidal crystals with nanometer precision that can be used to bend light in unnatural ways, we and others have significantly enhanced our understanding of chemistry and the natural world.

What the next 30 years has in store for this field is also impossible to predict, but some educated guesses can be made. A continued emphasis will likely be placed on chemical synthesis and characterization. Indeed, these areas are a cornerstone of colloidal crystal engineering with DNA, as they are in any area of chemistry. Fundamental understanding and capabilities are key drivers that advance both pure and applied science and engineering efforts. *An important question will be what other types of materials can one use to make PAEs with chemical precision and high purity, and what are their properties?* These materials will fill out the Table of PAEs and be used to strengthen the analogy of the nanoparticle "atom" and the DNA "bond". In addition, *how can one use these materials to make colloidal crystals of increasing complexity*[196]*—multicomponent, multifunctional, dynamic structures—where the location and type of every PAE and DNA bond within them is known?* Looking forward, protein cores, nature's nanoparticles, with well-known surface topologies will play a key role and

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dramatically expand structure control and properties that can be accessed.

Algorithms will be developed that allow one to design the particles and DNA required to make almost any arrangement of particles—a type of inverse design where materials can be made to exhibit a prespecified property. Once target PAEs or colloidal crystal systems are chosen, the issue of scale-up will need to be tackled. *How can PAEs and the corresponding colloidal crystals be prepared on a massive scale, while making sure that each structure made in the bulk retains all of the structural and functional features of the constructs that were prepared on a smaller scale?* This question will be important as colloidal crystal engineering with DNA is used to integrate PAEs into devices and tools. Such devices and tools will enable applications first embodied in proof-of-concept experiments spanning catalysis to optics to ultimately become backbone technologies that drive new fields and capabilities, as has already been done in biology and medicine with the prototypical building blocks. This is a call to action to nanoscientists from all areas, including chemistry, physics, engineering, medicine, materials science, and computer science, to join this effort in defining this emerging area of chemistry and applying principles within it to help solve some of society's most pressing problems.

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Notes

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