

Programming Supramolecular Biohybrids as Precision Therapeutics

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Conspectus: Chemical programming of macromolecular structures to instill a set of defined chemical properties designed to behave in a sequential and precise manner is a characteristic vision for creating the next generation nanomaterials. In this context, biopolymers such as proteins and nucleic acids provide an attractive platform for the integration of complex chemical design due to their sequence specificity and geometric definition which allows accurate translation of chemical functionalities to biological activity. Coupled with the advent of amino acid specific modification techniques, “programmable” areas of a protein chain become exclusively available for any synthetic customization. We envision that chemically reprogrammed hybrid proteins will bridge the vital link to overcome the limitations of synthetic and biological materials, providing a unique strategy for tailoring precision therapeutics.

In this Account, we present our work towards the chemical design of protein-derived hybrid polymers, their supramolecular interactions and responsiveness, as well as summarizing their impact and the advancement in biomedicine. Proteins, in their native form, represent the central framework of all biological processes and are an unrivalled class of macromolecular drugs with immense specificity. Nonetheless, the route of administering protein therapeutics is often vastly different from Nature's biosynthesis. Therefore, it is imperative to chemically reprogram these biopolymers to direct their entry and activity towards the designated target. As a consequence of the innate structural regularity of proteins, we show that supramolecular interactions facilitated by stimulus responsive chemistry can be intricately designed as a powerful tool to customize their functions, stability, activity profiles and transportation capabilities.

From another perspective, a protein in its denatured, unfolded form serves as a monodispersed, biodegradable polymer scaffold decorated with functional side chains available for grafting with molecules of interest. Additionally, we are equipped with analytical tools to map the fingerprint of the protein chain, directly elucidating the structure at the molecular level. Contrary to conventional polymers, these biopolymers facilitate a more systematic avenue to investigate engineered macromolecules, with greater detail and accuracy. In this regard, we focus on denaturing serum albumin, an abundant blood protein, and exploit its peptidic array of functionalities to program supramolecular architectures for bioimaging, drug and gene delivery. Ultimately, we seek to assimilate the evolutionary advantage of these protein based biopolymers with the limitless versatility of synthetic chemistry to merge the best of both worlds.

Introduction

Nature has been, in recent years, growing to be the prevailing model in directing the advent of modern science.^{1,2} With time, the role of Nature has evolved from an expansive pool of resources to become an inspiration, a golden standard, and in some ways, the Holy Grail.³ This is particularly evident in chemistry, as it is soon realized that chemical reactions within the biological system are energetically efficient, sustainable, highly specific and seemingly intelligent, all of which are still elusive in the synthetic world.² Looking at this great disparity and the bleak future of non-renewables, a great deal of interest has led to a rapid development in emerging areas ranging from small molecular green chemistry,⁴ biocatalysis¹ to self-assembly and higher ordered supramolecular architectures⁵ – all of which are autonomous processes in Nature.

Fundamentally, the molecular make up of these highly integrated systems are remarkably defined and coupled with atomic level recognition, each biological pathway becomes exceptionally specific.⁶ The overall observed functional diversity is intriguing, because the repertoire of small molecules that compose a living system is not particularly overwhelming as compared to the possibilities synthetic chemistry offers. Nonetheless, it is now understood that the workhorses of Nature that represent their far-reaching biological behavior rest on the macromolecular and supramolecular level, in which the customization of functions and activities are engineered or encoded from a small set of molecular libraries:⁷ It takes five nucleotides to code the genetic information necessary for proliferation and likewise in a protein scaffold, 20 amino acids connected via amide bonds in various permutations, lengths and post translational modifications are responsible for millions of proteins spanning the entire biological domain.⁶

Sequence Definition and Chemical Programming

Among major classes of biomolecules, proteins emerge prominently as versatile precursors to construct precision nanomaterials by providing a variety of chemical functionalities, defined sequences, architectures and functions while simultaneously complemented by site-specific mutagenesis techniques (Table 1). It is unanimously understood that the functions of proteins are derived from their architecture which is dictated by folding proteins building upon the supramolecular forces specifically programmed within the amino acid sequence.⁶ Each amino acid influences the next downstream monomer unit while complex through space interactions become crucial in contributing important physical and chemical properties like solubility, hydrophobic interactions, hydrogen bonding, disulfide bridges, metal chelation and α -helix/ β -sheet formation.⁶ Furthermore, these supramolecular forces are precisely defined for each protein and this epitome of chemical programmability, where molecular positioning defines the outcome in a regular, predictable and intelligent manner, is exemplified by how proteins behave in the biological system. Recognizing the importance of the sequence to customize physical or chemical characteristics, polymer chemists have made significant accomplishments in recent years by controlled polymerization methods to create precision polymers.⁸

While precision based polymer syntheses have been inspired by Nature, the biological system can also benefit from chemical tools to augment its capabilities and allow the reprogramming of the biomolecule (Figure 1).⁹ Importantly, the primary advantage of using a biological scaffold not only stands on its pre-defined sequence and monodispersity but rather how the molecule behaves chemically and physically as a result of such a molecular character.¹⁰ The folding of DNA, known as DNA origami, into various shapes and sizes that are not naturally found in Nature perfectly exemplify how a precision based material can be tailored as molecular motors¹¹

or nano-containers.¹² However, nucleic acids are functionally limited to create bioactive structures and as Nature evolved from nucleic acids to proteins as the foundation of biological processes, one could also do so likewise. Structurally, each protein is molecularly and macroscopically identical allowing chemical modifications of its functionalities in a predictable manner. Moreover, protein crystallographic data, computational modeling studies and peptide mass fingerprinting have provided invaluable information that are exploited routinely in chemical biology. Comparatively, the other classes of biomolecules (lipids, carbohydrates) as well as synthetic polymers are still at a disadvantage, mainly lacking in structural definition and does not provide as much utility in creating highly sophisticated yet precise functional assemblies (Table 1). As a result, utilizing proteins as a primary functional material has garnered increasing attention and reviewed extensively as polymer-protein hybrids^{13,14} or as protein based nanodevices.¹⁵ To instill a new perspective, we present that in achieving molecular order, the methodical incorporation of the massive repertoire of synthetic functionalities involving stimulus responsive (e.g. pH, light, temperature) and supramolecular chemistry can create a defined space for macromolecular engineering with biomedical relevance (Figure 2).

Tailoring Hybrid Globular Proteins as Macromolecular Solutions for Biomedicine

In the design of a macromolecular therapeutic, assigning various chemical components responsible for transport, activity or release is inevitable and by default, proteins themselves are able to fit in all three aspects as a result of their assigned roles in Nature. However, these unique biochemical functions of proteins have limited use unless they are reprogrammed with external chemical or biological intervention. Simplistically, endogenous proteins like the renowned tumor suppressor protein p53 do not innately possess cross-membrane transport, implying that an

externally delivered p53 protein has minimal chance of replacing a malfunction copy present in many tumor cells.¹⁶ Since proteins can, in principle, fulfill each aspect of a macromolecular therapeutic, it has been well known in biology to express fusion proteins through genetic manipulation for transport, activity and release.¹⁷ On the contrary, chemical tools to create unnatural or hybrid protein assemblies with complementary biological behavior are much less developed due to the intrinsic sensitivity of proteins, functional requirements and selection of aqueous chemistry.¹⁸

One of our strategies uses synthetic techniques to modify and customize the character of proteins to perform novel biological tasks that are consequently different from the native counterpart. For globular proteins, we have explored the use of small chemical functional groups to instill a variety of changes (charge distribution, stimulus responsiveness, supramolecular interactions) to proteins while maintaining their architectural precision (Figure 3). The alteration in the surface charges of proteins is a fundamental concept but like many biomaterials, the overall ionic character of proteins plays an important role in the biological context.¹⁹ Positively charged macromolecules such as cationic polymers/dendrimers are well known to interact with negatively charged membranes to promote cell adhesion and internalization.²⁰ However, they also possess inherent cytotoxicity when present at concentrations that disrupt the integrity of the cellular membrane and thus often require intricate balance between these two counteracting factors.²⁰ Alternatively, we show that this drawback can be significantly minimized by redistributing the charges onto a protein surface.²¹ Serum albumin (SA) was chosen as the target protein as it constitutes the major component in blood and provides natural biocompatibility whereas its repertoire of carboxylates, amines and a site-specific thiol group presents itself as an attractive platform for synthetic modifications. Furthermore, SA represents an ideal scaffold as a

drug delivery vehicle since it is enriched in tumour tissue due to the enhanced permeation and retention effects and its binding affinity towards lipophilic drug molecules have been extensively exploited in therapeutics i.e. ABRAXANE®.²² We investigate the cationization of SA primarily by two methods: a) Condensation of ethylene diamine onto the carboxylates of the protein,²¹ b) attachment of second generation poly(amido)amine (PAMAM) dendrons via copper catalyzed azide-alkyne cycloaddition (CuAAC).²³ These constructs showed improved cellular internalization, are non-cytotoxic at therapeutically relevant concentrations (<50 μM) and hence providing an impetus towards drug and gene delivery applications.^{21,23} Cationized bovine serum albumin (cBSA) demonstrated promising gene transfection capacity through complexation with the negatively charged phosphate groups of DNA (Figure 3a).²¹ Interestingly, even though ethylenediamine groups are sterically small, binding of fatty acids towards human serum albumin (HSA) was observed to take place at the cationized surface rather than within its binding sites.²⁴ Additionally, biofilms formed by the immobilization of cBSA showed outstanding compatibility in the fixation of lipid vesicles and whole cells.^{25,26} These cells remain viable and catalyze the enantio-selective reduction of ethyl acetoacetate to R-(-)ethyl-hydroxybutyrate in a microchannel reactor.²⁶

On the other hand, HSA modified by second generation PAMAM dendrons (DHSA) has a unique implication in its design. Structurally, they are different from cationized SA as the protein core is significantly enlarged (~ 2 nm) by the attachment of bulky dendrons to form a core-shell architecture.²³ Widely known in the field of polymer-protein hybrids, the design of a core-shell assembly provide the advantage of encapsulation of guest molecules as well as shielding the protein against sensitive environments and immune response.^{9,13,14} PAMAM dendrimers, positively charged at physiological pH due to the presence of multiple primary amine groups on

their periphery, are known to facilitate cellular uptake.²⁷ Similar to native albumin, PAMAM dendrimers have previously shown to be capable of binding lipophilic drug molecules (e.g. paclitaxel, doxorubicin) in a non-covalent fashion within internal cavities.^{28,29} As a result, the protein core and the dendron shell act synergistically to accomplish a much higher drug loading capacity and increased uptake compared to native HSA into cells, significantly reduced carrier toxicity and lastly, increased potency of the cargo drug (Figure 3b).²³ Besides adopting covalent chemistry for the construction of precision biohybrids, supramolecular strategies are appealing to introduce a set of innovative tools and programmable functions into the protein scaffold. In Nature, these non-covalent interactions are often transient where formed complexes are non-permanent and exist as a response to a cellular signal or stimulus (i.e. glycogen). Collectively, these supramolecular forces constitute the smart interactive network of biological processes and regulation of these interdependent pathways. As a result, beyond the multitude of functionalities on proteins, specific protein/protein or protein/substrate interactions can be exploited to create responsive protein based nanomaterials.

(Strept)avidin, two widely known tetrameric proteins that bind biotin with high affinity ($\sim 10^{15}$ M⁻¹), have seen widespread applications in fusion proteins and antibody therapeutics.³⁰ With four binding sites available on each protein, we show that an assembly of biotinylated p53 tumor suppressor protein and biotinylated G2 PAMAM dendrons onto the (strept)avidin core can be achieved with high structural definition.³¹ Despite this combinatorial strategy enabling rapid *in vitro* evaluation of exogenous proteins, the exact ratio of dendrons and the cargo remains statistically varied on a molecular level.³¹ This flaw can be alleviated by exploiting the pair-wise diametrical position of the biotin binding sites where, on a solid support, one pair can be consequently blocked from the binding event (Figure 3c). In addition, a pH responsive analog of

biotin, iminobiotin, is introduced into the macromolecular design to instill a level of dynamic character for conditional release.³² Iminobiotin contains an imine functionality in place of the carbonyl group of biotin and as a consequence, its binding towards avidin is regulated by its extent of protonation (pH 11: $K_d \sim 10^{-8}$ M, pH 4: $K_d \sim 10^{-2}$ M).³³ Hence, on an iminobiotin functionalized surface, avidin molecules are immobilized under basic conditions and in the sequential step, a biotinylated transporter protein is loaded onto one of the two exposed binding site with steric control. This precise heterodimeric avidin-transporter construct is subsequently eluted at pH 4, revealing the binding site previously blocked by the solid support. In the final step, an iminobiotinylated protein cargo (β -galactosidase/C2I botulinum toxin) can be docked specifically into place to furnish a discrete supramolecular hetero-trimeric system that also facilitates cargo release through the acid responsive iminobiotin linker.³² Atomic force microscopic imaging and fluorescence correlation spectroscopy (FCS) demonstrate the exact macromolecular dimensions of this modular construct and *in vitro* assays exemplify that each individual protein constituent retain their biological function.³² As such, this method showcase a chemical equivalent of a fusion protein that provides a platform for organizing hybrid or chemically modified proteins in a precise manner, particularly attractive in the field of chemical biology.

Intuitively, one could substitute bio-based supramolecular interactions with synthetic groups to allow higher chemical flexibility and structural variability in the construction of hybrid materials. Cucurbituril, cyclodextrin, metal-ligand complexation and rotaxanes represent some of the prominent supramolecular building blocks which have been used to create precise structural materials based on molecular recognition.³⁴ Recently, boronic acids have begun to establish a foothold in this area due to its specificity towards 1,2 and 1,3-diols to form responsive cyclic

esters.^{35,36} Since then, investigation of these boronic acid ligands has evolved from protecting group chemistry for organic synthesis to supramolecular chemistry with base labile methyliminodiacetic acid and acid labile catechols or salicyl hydroxamates.³⁶ Adopting the core shell dendritic design based on our polycationic system, we selected and incorporated the boronic acid/salicyl hydroxamate recognition into the hybrid protein scaffold to further explore the concept of responsive protein based therapeutics (Figure 3b).³⁷ With facile supramolecular strategies, covalent reactions that would exert a detrimental effect on enzyme activities can be minimized. In this case, boronic acid moieties are introduced to reprogram the surface of enzymes (trypsin, papain, DNase I) so that it spontaneously assembles at neutral pH a dendritic shell made up of second generation PAMAM dendrons each possessing a salicyl hydroxamate core group. These spatially large macromolecules protect and isolate each enzyme from the surrounding, thereby preventing substrate entry and its resulting catalytic activity.³⁷ As the boronic acid/salicyl hydroxamate complexation is labile at pH 5.0, the dendritic shell can be dissociated at acidic conditions and in the process the active enzyme is released. Translating into biological significance, these assembled dendritic enzymes were shown to internalize into A549 cells due to their cationic character and localized into acidic compartments as part of the endocytosis pathway.³⁷ The active enzymes were spontaneously released within the cell and rampant cell death from the enzymatic activity was observed.³⁷ Notably, the process from which the supramolecular construct was assembled until its release within the cell, we present a system introducing simple yet effective programmable functions into chemically coded protein therapeutics.

Denatured Proteins as Precision Polypeptides

In the previous sections, we have established that proteins, in their globular form, are exemplary candidates for synthetic modifications, adding diversity to their roles as an emerging class of macromolecular therapeutics. Can proteins achieve a broader scope of applications within the biomedical setting? Conceivably through chemical techniques, we can extend our leverage on both the polypeptide sequence and its supramolecular interactions in a controlled and systematic fashion to create unique functional materials. When devoid of its globular structure, the multifunctional polypeptide presents a natural modification platform parallel to that of post-polymerization techniques in synthetic polymer chemistry. The conceptual introduction of denatured proteins as a primary polymeric scaffold for the design of biocompatible materials was first discussed by Whitesides et al.³⁸ Possessing a precise length and absolute monomer positioning along the polymer chain, these protein-derived polypeptides provide a superior platform to program and attribute self-assembly behavior with higher accuracy (Figure 4).¹⁰ Synthetically, the denaturation process of a protein into its unfolded form is commonly associated with aggregation problems due to the exposure of hydrophobic amino acid residues that would otherwise be stabilized within the core of a globular protein.³⁸ Conceptually, the use of sterically large solubilizing moieties would consequently mediate the hydrophobicity of the polypeptide and disrupt undesirable supramolecular interactions between denatured protein chains. In this aspect, poly(ethylene)oxides (PEO) are attractive candidates both from a chemical and biological perspective. These synthetic polymers are widely established for their hydrophilicity capable of significantly increasing the hydrodynamic radius of a material of interest, interrupt protein-protein interactions and inert to physiological conditions.¹³ As a consequence, PEOs are non-toxic, enhance blood circulation due to increase in apparent size of a

conjugated molecule as well as conferring “stealth” properties by sterically masking immunogenic recognition sites of foreign materials.³⁹ Exploiting these beneficial biological properties, we covalently linked poly(ethylene)oxides-2000/5000 (PEO₂₀₀₀/PEO₅₀₀₀) to the reduced cysteine residues with thiol-maleimide chemistry.⁴⁰ This conjugate addition strategy was preferentially chosen in order to compensate the accessibility of reduced cysteines and the sterically demanding PEOs with the efficiency of the reaction. Hindered thiol functionalities located in the immediate vicinity of a PEO modification are capped with smaller amino-maleimides to prevent aggregation processes promoted by disulfide reformation.

Intuitively, the covalent conjugation of PEOs can take place on other amino acid residues i.e. lysines that are usually located at the surface of the globular protein.⁴¹ Subsequent protein denaturation still provides a full range of chemical moieties along the polypeptide main chain as the cysteines that were buried within the core are now exposed for further chemical modifications.⁴¹ By taking advantage of protein conformation in determining its chemical reactivity, we can further augment the engineering potential of each functional group within the protein sequence. This convenient method of preparing unfolded protein polymers facilitating further chemical modifications has been applied to proteins of different sizes (e.g. human serum albumin (HSA): 66 kDa, hen-egg white lysozyme: 14 kDa).⁴² As a result of these PEO attachments, the resultant single chain protein-polymer hybrids exhibited a larger hydrodynamic size compared to the native proteins and the secondary structures displayed a slight increase in the proportion of random coils compared to α -helices.⁴² Biological evaluation of these hybrid polypeptides showed that positive charge densities along the backbone correlate to an increase in cellular uptake similar to that of globular modified proteins. Moreover, the hydrophobic cavities originated from albumin for transporting lipophilic ligands were also found partially retained in

these polypeptides to promote supramolecular interactions.²⁴ Furthermore, derived from proteins ubiquitously found, these polypeptides are non-toxic, biodegradable, possess excellent solubility and stability in aqueous media thus facilitating applications in nanomedicine.⁴² With these prominent characteristics, we envisioned that such single chain polypeptide polymers with grafted PEO chains could be designed to address problems involving toxicity, biocompatibility, aqueous chemistry, solubility and stability of synthetic materials (Figure 4b).

In particular, contemporary materials such as carbon nanotubes,⁴³ quantum dots (QD),⁴⁴ and nanodiamonds (ND)⁴⁵ are often restricted by some of the aforementioned disadvantages that limit their applications towards imaging and cancer treatment. As a proof of concept, the denatured HSA-PEO polymer was additionally decorated with thioctic acid moieties and their subsequent reduction allow the coating of the polypeptide onto CdSe-CdZnS quantum dots.⁴⁰ These coated counterparts are stable in water for several months and revealed a pronounced pH dependent fluorescence behavior as a result of changes of charge densities and secondary structures of the protein coating (Figure 5a). Transmission electron microscopy (TEM) and dynamic light scattering (DLS) on these coated quantum dots at different pH verified that the observed fluorescence response is not a consequence of aggregation.⁴⁰ In addition, we show that these coated quantum dots are efficiently uptaken into cells and biocompatible.⁴⁰ Through live cell FCS, the diffusion behavior of individual quantum dots was visualized in endosomes providing an invaluable tool to study the physical properties and biological behavior of these fluorescent hybrids within the cell. In addition, if a cationized denatured HSA (cHSA) was used to alter the electrostatic map of the quantum dot, DNA complexation could be achieved.⁴⁶ In this case, photoluminescent properties of the quantum dot were shown to decrease upon its complexation with DNA resulting in an optical display of parts of the DNA transfection process

(Figure 5b).⁴⁶ Chemically, one could immediately see that the use of a denatured protein backbone where each set of functional amino acids at defined positions can be categorically designed to possess a discrete set of synthetic properties is extremely compelling when compared to synthetic polymers.

Since these newly integrated functions possess explicit molecular positioning, we seek to evaluate and map the supramolecular assembly behavior for these denatured protein hybrids to systematically understand the implication of each modified entity. Through AFM and DLS techniques, the library of denatured proteins possessing varying charge densities, length, lipophilicity contribution by thioctic acid modifications were investigated.⁴⁷ Discrete nanoscale and mesoscale assemblies as a result of these modifications were achieved reflecting their molecular homogeneity and the effect of chemical functionalization towards hierarchical self-assembling behavior. The resultant nanostructured films were bioactive and even stable to post assembly modification, which could become exceptionally useful in creating tailored compatible coatings that influence critical biological characteristics i.e. the adhesion or proliferation of stem cells.

As the use of synthetic chemistry allows the morphology and supramolecular interactions of these denatured proteins to be tailored, we could apply this technology to create defined nanoenvironments for catalysis,⁴⁸ drug delivery⁴¹ and as programmable hydrogels (Figure 5c, d).⁴⁹ Since lipophilic domains within the denatured backbone have shown to enable the collapse of the denatured protein backbone, reaction efficiencies could be enhanced within these confined spaces.⁴⁸ High labelling efficiency of doxorubicin was observed and the resultant protein-polymer-drug conjugate displayed toxicity comparable to the unbound drug molecule.⁴⁸ Following this, we seek to incorporate stimulus responsive chemistry into the polypeptide chain

to enable greater control and selectivity of its pharmacokinetic properties. Hence, we replace the covalent triazole linking doxorubicin to the denatured protein backbone with acid-labile hydrazone chemistry to program its dissociation inside acidic intracellular compartments.⁴¹ In order to stimulate drug release, the scaffold would have to be degraded within the cell and consequently adopt a two-step release profile (Figure 5d).⁴¹ Unlike synthetic polymers, this polypeptide based carrier is sensitive towards proteolytic enzymes such as cathepsin B which is highly expressed within tumor cells.⁵⁰ In a detailed drug release study, we showed that the presence of cathepsin B as a co-stimulus resulted in a very favorable release of >70% of the conjugated drug molecules after 48 h.⁴¹ *In vitro* cellular evaluation of the denatured protein-drug micelles on various cell lines including human acute myelocytic leukemia cells showed significant potency with an IC₅₀ as low as 1.9 nM. Additionally, effective shielding of epitopes due to the PEG chains were demonstrated by ELISA, which would be valuable in reducing the immunogenicity of the hybrid co-polymer.⁴¹ Consequently, the hybrid system tested *in vivo* on NSG mice via leukemic engraftment demonstrated 100% survivability after 84 days.⁴¹ From both structural and biological perspective, these reprogrammed protein transporters offer a biocompatible shell, versatile surface as well as interior chemistry and these attributes distinguish these materials from pure polymer transporters or liposomes.

Besides utilizing supramolecular forces to create restructured globular hybrid materials, the same can be extrapolated to create biocompatible and responsive hydrogels for the controlled release of functional proteins (Figure 5c).⁴⁹ Orthogonal single stranded DNA (ssDNA) sequences are conjugated onto the cHSA-PEO hybrid in which gelation can be achieved using complementary three or four-armed DNA. Two arms are necessarily needed for crosslinking with the extra arms hybridized with GFP/YFP and the eventual hydrogel facilitates an enzymatic

triggered release of the target protein.⁴⁹ In this case, the advantage of using exclusive DNA base-pairing and oligonucleotide design allow us to further increase the complexity of the denatured cHSA-PEO hybrid in a systematic fashion. In particular, customization of functionalities can subsequently be pushed beyond selective chemical modifications of the polypeptide backbone into DNA hybridization techniques without compromising on molecular precision.

Collectively, these results establish new frontiers in macromolecular science where synthetic chemistry is recognized to play a pivotal role as an innovative tool to impart unique supramolecular behavior and stimulus responsive characteristics into an integrative platform. Although primary investigations involving recognition by antibodies showed promise, the immunogenicity profile of these hybrid materials is an important aspect that would require critical evaluation to achieve full biocompatibility.

Outlook

In summary, we have presented in this Account some of our strategies that offer a unique perspective of using proteins as a precision biomaterial for macromolecular engineering. By employing an elaborate array of chemical techniques, we have investigated how polyelectrolyte, bioorthogonal, responsive and supramolecular chemistry can be utilized to reprogram protein functions and capabilities for biomedical applications. From the fundamental aspects of monodispersity and sequence definition of proteins, synthetic transformations can be tailored at molecular accuracy in a combinatorial fashion to afford highly ordered bioarchitectures. With each available protein sequence simultaneously presenting such a potential, one could imagine a significant advantage over synthetic polymers based on these chemical differences alone. Despite the ease to have a sequence elucidation of protein based materials and create defined end-

products, the connection between molecular functionality on a supramolecular level remains complex due to the presence of other chemically saturated amino acid residues i.e. phenylalanine, glycine, leucine. Another limitation of using protein based materials would be the invariant nature of the polypeptide chain. Although amino-acid based mutagenesis allows customization towards the polypeptide, proteins that are extensively mutated are spontaneously degraded upon expression as they are recognized as defective by the cellular translation machinery.

Nonetheless, the macromolecular properties of proteins and their relevance towards the development of contemporary biomedicine are glaring. Furthermore, in combination with other state-of-the-art assembling methods such as layer-by-layer⁵¹ or Langmuir-Blodgett techniques,⁵² the possibilities to create and customize precision nanodevices would be immense. With modern chemical techniques, the integration of synthetic programmable functionalities with the structural precision of proteins have demonstrated great promise as a unique class of macromolecules that can be tailored to complement the biological system. As such, it could be envisioned that these hybrid systems may initiate an evolutionary change in the administration of medicine and the future of nanomaterial development.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

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ABBREVIATIONS

AFM, atomic force microscopy; DLS, dynamic light scattering; PEO, poly(ethylene)oxide; DNA, deoxyribonucleic acid.

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FIGURES

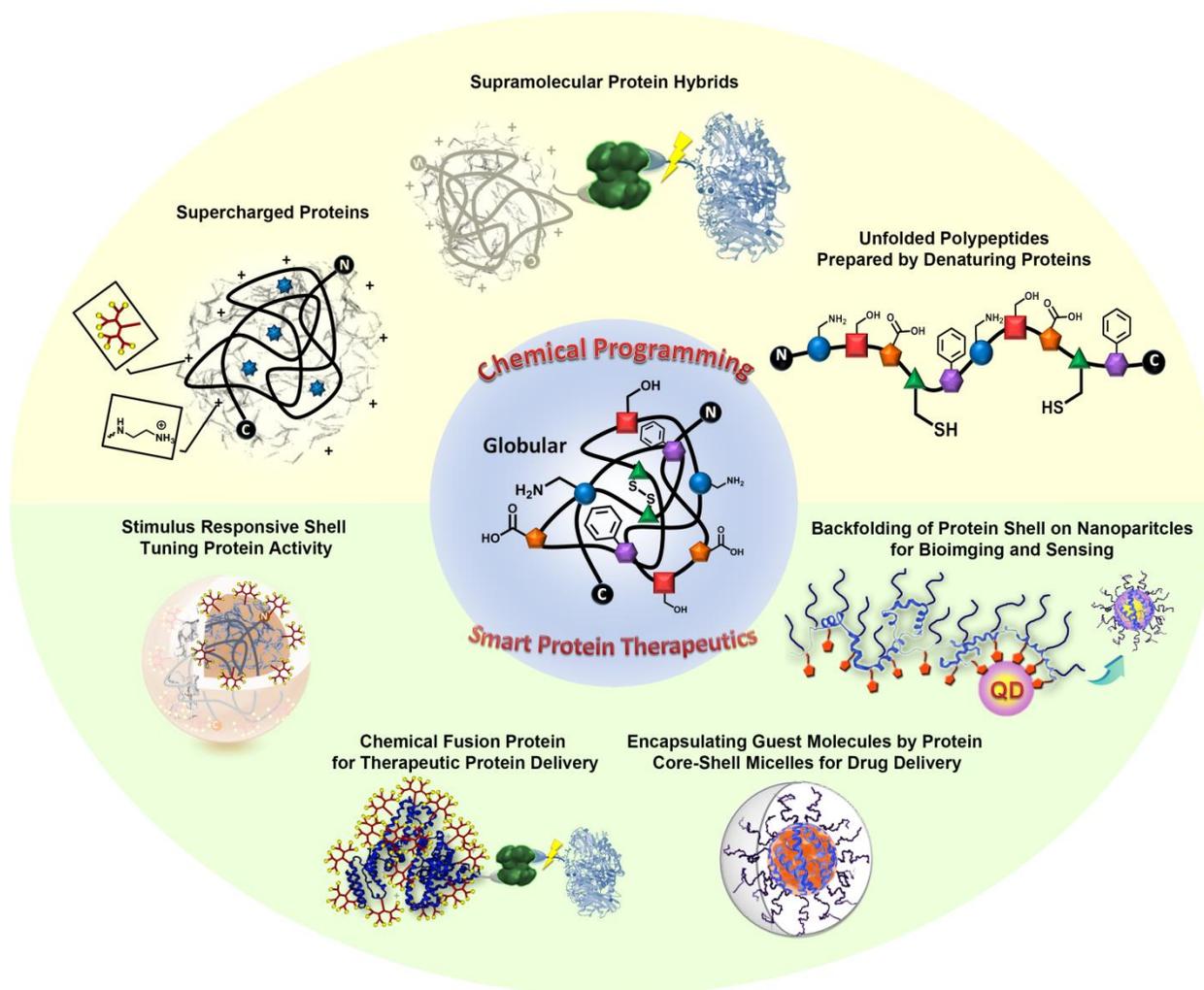


Figure 1. Overview of chemically programmed protein based biohybrids as macromolecular therapeutics

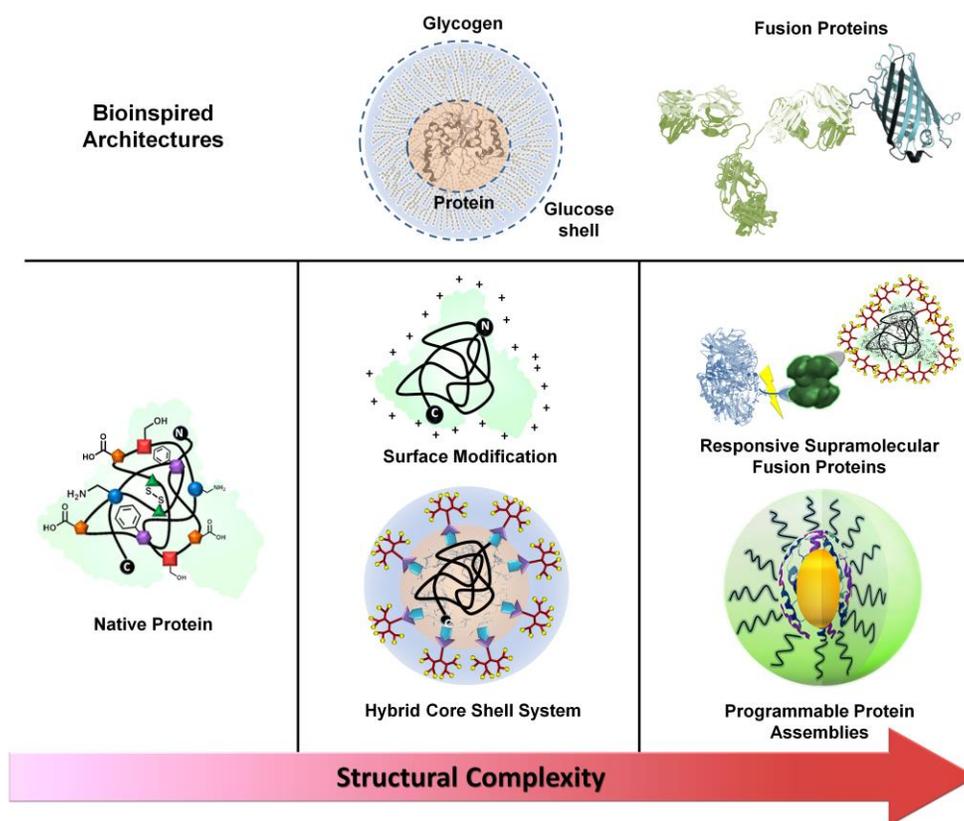


Figure 2. Progressive design of precision bioarchitectures for therapeutic delivery and responsive release.

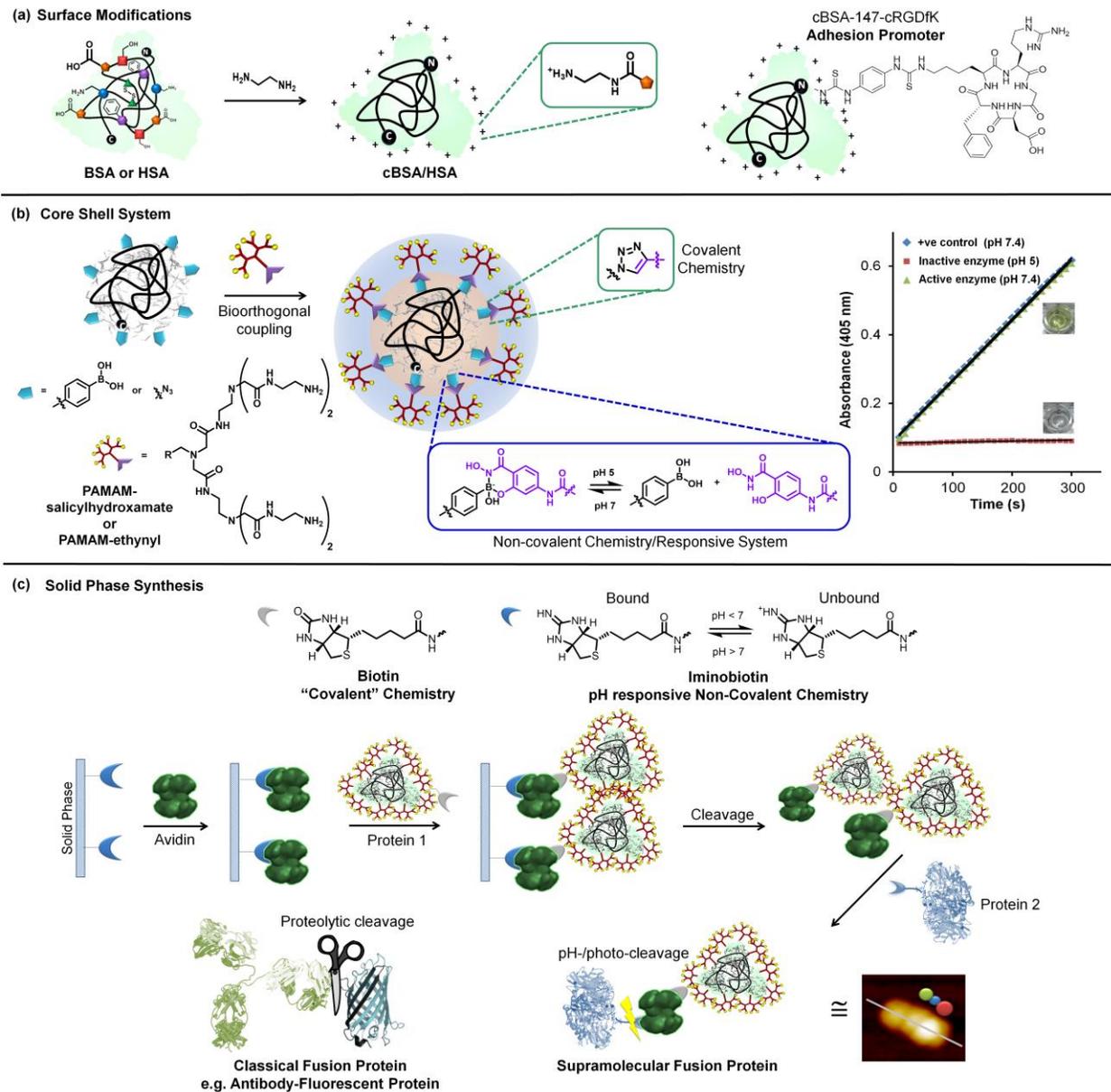


Figure 3. (a) Modification of surface charges of serum albumins for drug and gene delivery. (b) Covalent and supramolecular core shell hybrids. (c) Solid phase immobilization strategy to derive responsive chemical fusion proteins.

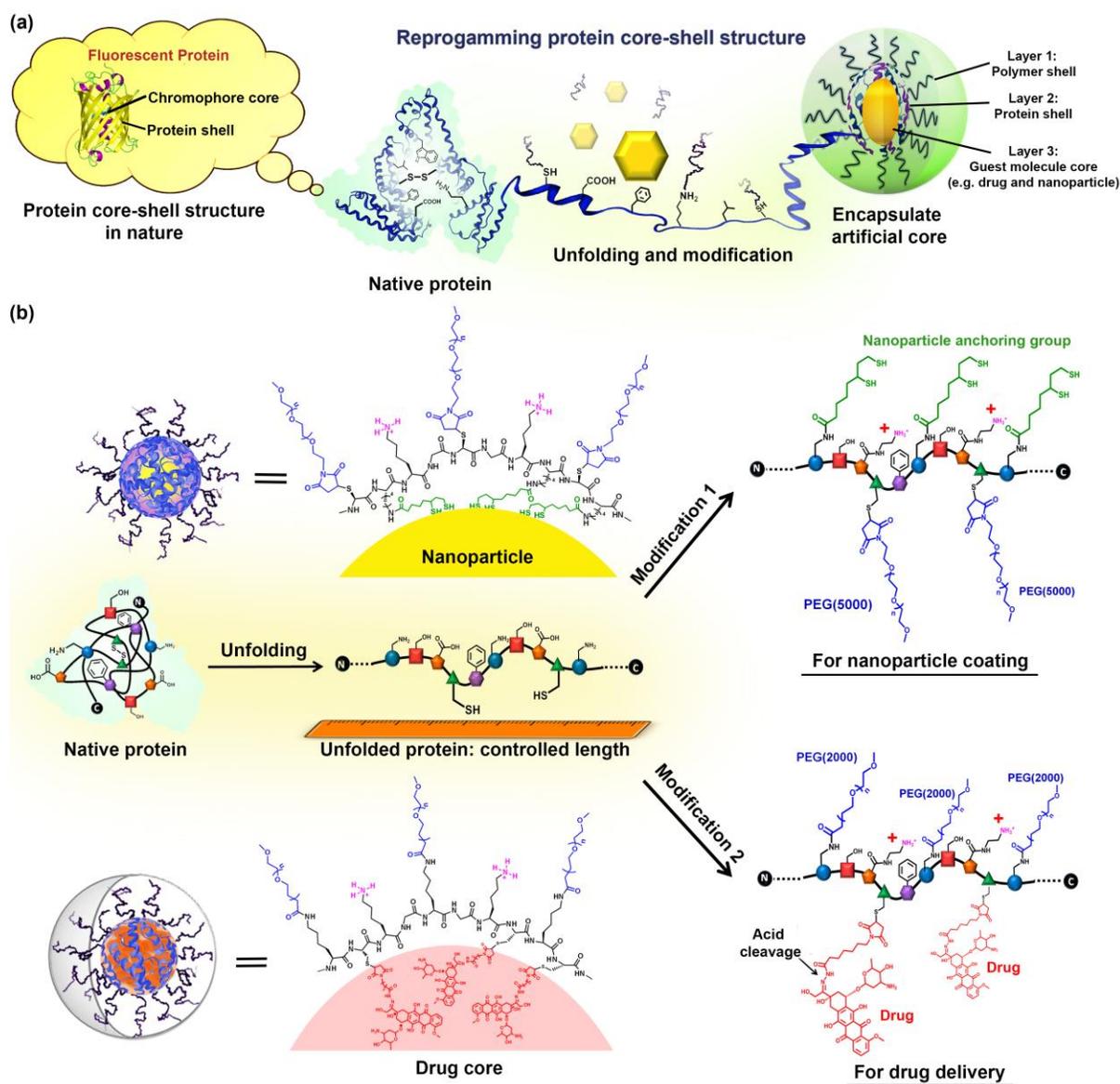


Figure 4. (a) Chemical induced unfolding of a globular protein into denatured protein-PEO hybrid and backfolding of globular structure induced by nanoparticles and hydrophobic guest molecules. *The term of “backfolding” does not imply refolding of the secondary or tertiary structure of the native protein but it refers to the spontaneous assembly of the polypeptide chain into a globular architecture. (b) Chemical programming of denatured protein polypeptide using orthogonal modifications on different sets of amino acids.

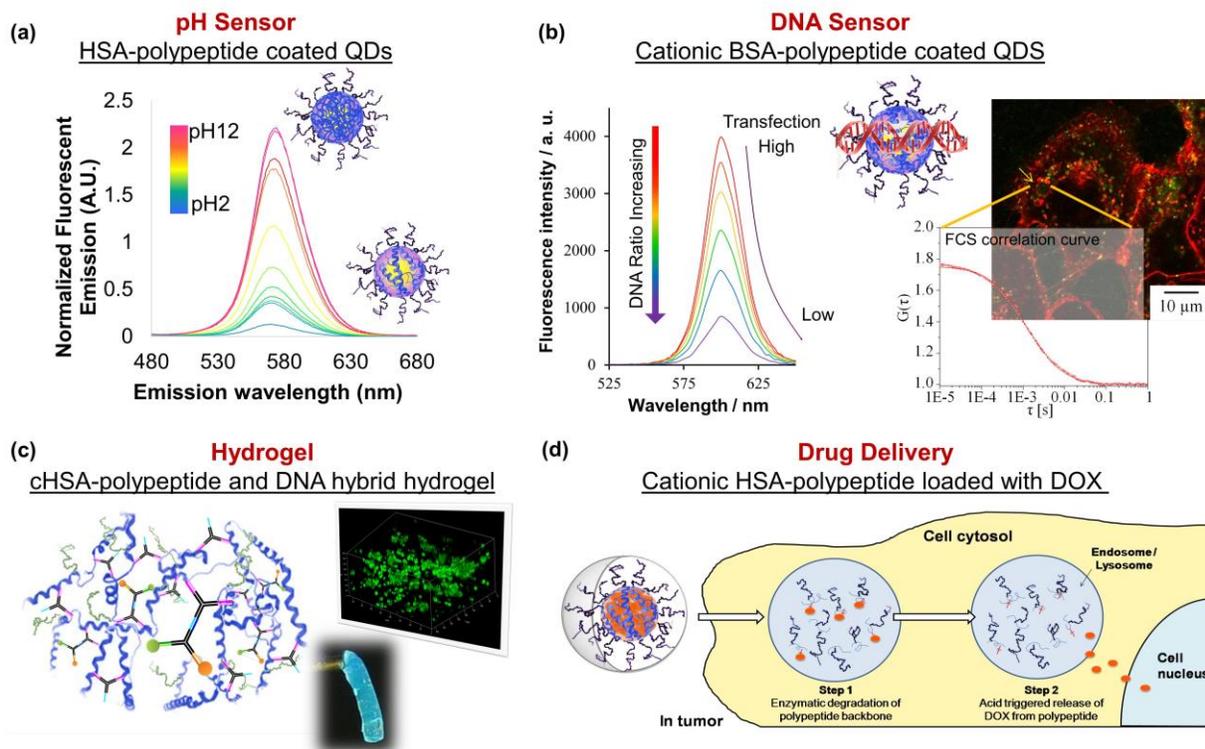


Figure 5. (a) Denatured HSA-PEO hybrid coated quantum dots as pH sensor. The secondary structures of denatured HSA-PEO hybrid change under different pH as a result of altered passivation on the QDs surface. (b) Live cell FCS imaging of cBSA-PEO coated QDs and fluorescence monitoring of gene transfection. (c) Denatured cHSA-PEO hybrid with DNA cross-linked hydrogel for responsive release of active proteins. (d) Denatured cHSA-PEO hybrid forming a core-shell drug delivery carrier for two-step controlled drug delivery into tumor cells.

	Reactive Groups	Precision/Dispersity	Functional Activity	Biocompatibility*	Customization
Proteins & Peptides	Amino, Carboxyl, Hydroxyl, Sulfhydryl, Phenolic, Indole	Defined	Structural, Catalytic, Transport	Full	Site Specific Mutagenesis, Precision Modification Peptide Aptamer
Nucleic Acids	Hydroxyl, Phosphate	Defined	Structural, Catalytic (Limited), Transport	Full	DNA Origami DNA Aptamer
Carbohydrates	Hydroxyl	Branch Variation	Structural	Full	Post Modification
Lipids	Carboxyl, Hydroxyl, Phosphate	Defined (Molecular) Vary (Liposomes)	Structural (Micelles, Vesicles), Transport	Good	Flexible Monomer Design
Polymers	Unlimited	Narrow (Controlled Living Polymerization)	Structural, Catalytic (emerging), Transport (preliminary)	Limited	Flexible Monomer Design

*Immunogenicity is excluded and discussed in the text

Table 1. Characteristics of major classes of biomolecules and polymers as a primary platform for macromolecular design.

CONSPECTUS GRAPHIC

