SUPPORTING INFORMATION

DNA-Encoded Protein Janus Nanoparticles

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Materials and Methods

S1. Protein mutation, expression, and purification

S1.1. EGFP mutagenesis

Site-specific mutagenesis of the EGFP-pBAD vector (EGFP-pBAD was a gift from Michael Davidson, Addgene plasmid #54762) was achieved using a QuikChange II Site-Directed Mutagenesis Kit (Agilent). This technique employs PCR-thermocycling reaction using mutagenic primers, followed by removal of the original plasmid template by *Dpn*I digestion. Mutagenic primers were designed to introduce a single codon mutation into the gene. To facilitate annealing, primers contained at least 40% GC, had melting temperatures (Tm) above 60 °C, and exhibited one or more Gs and Cs at the 5′ or 3′ ends (Table S1). Two consecutive mutations were performed to convert the surface serine (S148) to a cysteine (C148) and also mutate an unwanted, partially surface accessible cysteine (C49) to a leucine (L49). Successful mutations were confirmed using traditional Sanger Sequencing (Table S2).

Table S1. Mutagenetic primer design of S148 to C148 mutation.

Primer	Sequence (5′ to 3′)	GC content	Tm / °C
S148 → C148 (A)	TGA TAT AGA CGT TGT GGC AGT TGT AGT TGT ACT CCA G	43.2 %	62.5
S148 → C148 (B)	CTG GAG TAC AAG TAC AAC TGC CAC AAC GTC TAT ATC A	43.2 %	62.5

Table S2. Sanger sequencing data of mutated EGFP plasmid compared to native plasmid.

	Protein Sequence
Native	M V S K G E E L F T G V V P I L V E L D G D V N G H K F S V S G E G E G D A T Y G K L T L K F I C T T G K L P V P W P T L V T T L T Y G V Q C F S R Y P D H M K Q H D F F K S A M P E G Y V Q E R T I F F K D D G N Y K T R A E V K F E G D T L V N R I E L K G I D F K E D G N I L G H K L E Y N Y N S H N V Y I M A D K Q K N G I K V N F K I R H N I E D G S V Q L A D H Y Q Q N T P I G D G P V L L P D N H Y L S T Q S A L S K D P N E K R D H M V L L E F V T A A G I T L G M D E L Y K
C49 to L49	M V S K G E E L F T G V V P I L V E L D G D V N G H K F S V S G E G E G D A T Y G K L T L K F I **L** T T G K L P V P W P T L V T T L T Y G V Q C F S R Y P D H M K Q H D F F K S A M P E G Y V Q E R T I F F K D D G N Y K T R A E V K F E G D T L V N R I E L K G I D F K E D G N I L G H K L E Y N Y N S H N V Y I M A D K Q K N G I K V N F K I R H N I E D G S V Q L A D H Y Q Q N T P I G D G P V L L P D N H Y L S T Q S A L S K D P N E K R D H M V L L E F V T A A G I T L G M D E L Y K
S148 to C148	M V S K G E E L F T G V V P I L V E L D G D V N G H K F S V S G E G E G D A T Y G K L T L K F I **L** T T G K L P V P W P T L V T T L T Y G V Q C F S R Y P D H M K Q H D F F K S A M P E G Y V Q E R T I F F K D D G N Y K T R A E V K F E G D T L V N R I E L K G I D F K E D G N I L G H K L E Y N Y N **C** H N V Y I M A D K Q K N G I K V N F K I R H N I E D G S V Q L A D H Y Q Q N T P I G D G P V L L P D N H Y L S T Q S A L S K D P N E K R D H M V L L E F V T A A G I T L G M D E L Y K

S1.2. Protein expression and purification

The mutated plasmid containing the gene for the mutated EGFP was transformed into One Shot®
BL21(DE3) Chemically Competent E. coli (Thermo Fisher) by heat shock, and cells were grown
overnight on LB Agar plates with 100 μg/mL ampicillin. Single colonies were picked, and 7 mL
cultures were grown for 6 h at 37 °C in LB broth with 100 μg/mL Ampicillin. These cultures were
added to 1 L of Terrific Broth (Thermo Fisher) with 1 % glycerol and 100 μg/mL ampicillin, and cells
were grown at 37 °C to an optical density of 0.6, then induced with 0.02 wt% arabinose overnight at
17 °C. Cells were spun down (6000 g, 30 min) and resuspended in 100 mL of 1x PBS, then lysed

using a high-pressure homogenizer. The cell lysate was clarified by centrifugation at 30,000 g for 30 min and loaded onto a Bio-Scale™ Mini Profinity™ IMAC Cartridge (Bio-Rad). The column was washed with 100 mL of resuspension buffer, then eluted in the same buffer with 250 mM imidazole. The eluted fraction was immediately exchanged 5 times into 1x PBS using 30 kDa molecular weight cut-off filters (Millipore). After concentrating to 3 mL, the protein was further purified using a size exclusion column (SEC650, Bio-Rad), and fractions with molecular weights corresponding to mutant EGFP (mEGFP) were stored at 4 $^{\circ}$C for up to two months.

S2. Oligonucleotide design and synthesis

S2.1. Oligonucleotide design
Six orthogonal oligonucleotide sequences were designed specifically for the protein Janus particles. Amine terminated 18 and 24 base-pair sequences, as well as their respective complementary strands, were designed to form interprotein DNA bonds of different lengths. Two dibenzocyclooctyne (DBCO) functionalized strands, containing orthogonal sequences, were used to decorate each domain of the Janus particle.

A further two orthogonal sequences were designed for the functionalization of the inorganic nanoparticles: of which, both thiol and dithiol-modified versions were prepared. Four linker strands were required: one complementary strand for each domain of the protein Janus particle, as well as the corresponding complimentary strands for orthogonally functionalized inorganic nanoparticles.

S2.2. Oligonucleotide synthesis
All oligonucleotides were synthesized on solid supports using reagents obtained from Glen Research and standard protocols (Table S3). Products were cleaved from the solid support using 30% NH_3 (aq) for 16 hours at room temperature and purified using reverse-phase HPLC with a gradient of 0 to 75 % acetonitrile in triethylammonium acetate buffer over 45 min. The masses of the oligonucleotides were confirmed using matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) using 3-hydroxypicolinic acid as a matrix. All masses were within 20 Da of the expected calculated mass. Oligonucleotide concentration was calculated based on the extinction at 260 nm, using the extinction coefficients given in Table S3 that were calculated using the IDT Oligo Analyzer tool.

Table S3. Oligonucleotide sequences designed for the assembly of inorganic nanoparticles with DNA-encoded protein Janus nanoparticles. Extinction coefficients calculated using IDT OligoAnalyser Tool. Molecular weight (MW) measured using MALDI spectroscopy.

Name	Sequence (5′ to 3′)	ε (M^{-1}cm^{-1})	MW (Da)
DNA-Encoded Janus Particles			
Interprotein Bond 18 BP	**Amino C6**-AGT TAG GAC TTA CGC TAC	176900	5680
Interprotein Bond 18 BP	**Amino C6**-GTA GCG TAA GTC CTA ACT	177100	5682
Interprotein Bond 24 BP	**Amino C6**-AGT TAG GAC TTA CGC TAC TTT TTT	225200	7503
Interprotein Bond 24 BP	**Amino C6**-AAA AAA GTA GCG TAA GTC CTA ACT	250600	7557
DBCO-modified C	**DBCO-Sp$_2$**-GAA TAT TGA CGT AAA TCT	185100	6780
DBCO-modified D	**DBCO-Sp$_2$**-CAA TTA AAG TCA TAC AGA	191800	6766
Inorganic Nanoparticles			
Thiol-modified A	**C6 SS-Sp$_2$**-AAC GAC TCA TAT TAA CAA	188300	6477
Thiol-modified B	**C6 SS-Sp$_2$**-AAG ACG AAT ATT TAA GAA	200500	6578
Cyclic disulfide modifed A	**DS-Sp$_2$**-AAC GAC TCA TAT TAA CAA	188300	6561
Cyclic disulfide modified B	**DS-Sp$_2$**-AAG ACG AAT ATT TAA GAA	200500	6664
Linker Strands			
Linker A	AAGGAA-**Sp**-TTG TTA ATA TGA GTC GTT	248200	7785
Linker B	GAAAGA-**Sp**-TTC TTA AAT ATT CGT CTT	237700	7682
Linker C	TTCCTT-**Sp**-AGA TTT ACG TCA ATA TTC	227000	7616
Linker D	TCTTTC-**Sp**-TCT GTA TGA CTT AAT TG	218000	7619

Sp = hexaethylene glycol spacer unit

C6 SS = 5′ C6 thiol modifier

DS = 5′ Dithiol serinol modifier

S3. Synthesis and characterization of DNA-encoded Janus particle

DNA-Encoded Janus particles were prepared in a step-wise fashion. Each domain (A&B) of the Janus

particle was prepared separately, in parallel, using orthogonal oligonucleotide sequences. Janus

particles containing either an 18 or 24 base-pair DNA "bond" were synthesized using identical procedures.

S3.1. DNA conjugation to surface cysteine

Amine terminated DNA (300 nmoles) was reacted with 50 equivalents of SPDP (Figure S1 Thermo Fischer Scientific) crosslinker in 50 % DMF, 1x PBS + 1 mM EDTA for 1 h at room temperature. Excess SPDP was removed from the DNA by two rounds of size exclusion using NAP10 and NAP25 columns (GE Healthcare) equilibrated with PBS (pH 7.4), consecutively. Ten equivalents of the resulting pyridyl disulfide terminated DNA was added to 1.5 mL of 20 μM protein solution, and the reaction allowed to proceed for 16 h at room temperature. Excess DNA was removed from the reaction by 5 rounds of centrifugation in 30 kDa molecular weight cut-off filters (Millipore).
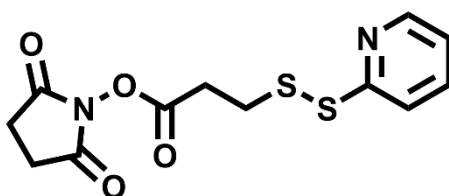

Figure S1. Structure of NHS-ester-pyridyl disulfide (SPDP) linker used to prepare pyridyl disulfide terminated DNA.

Anion exchange chromatography was performed on the mEGFP-DNA conjugate, using Macro-Prep® DEAE Resin, to remove any unreacted protein. SDS-PAGE analysis (Figure S2) confirmed elution of unreacted protein and pure mEGFP-DNA conjugate in buffers containing 0.25 M and 0.50 M NaCl, respectively.
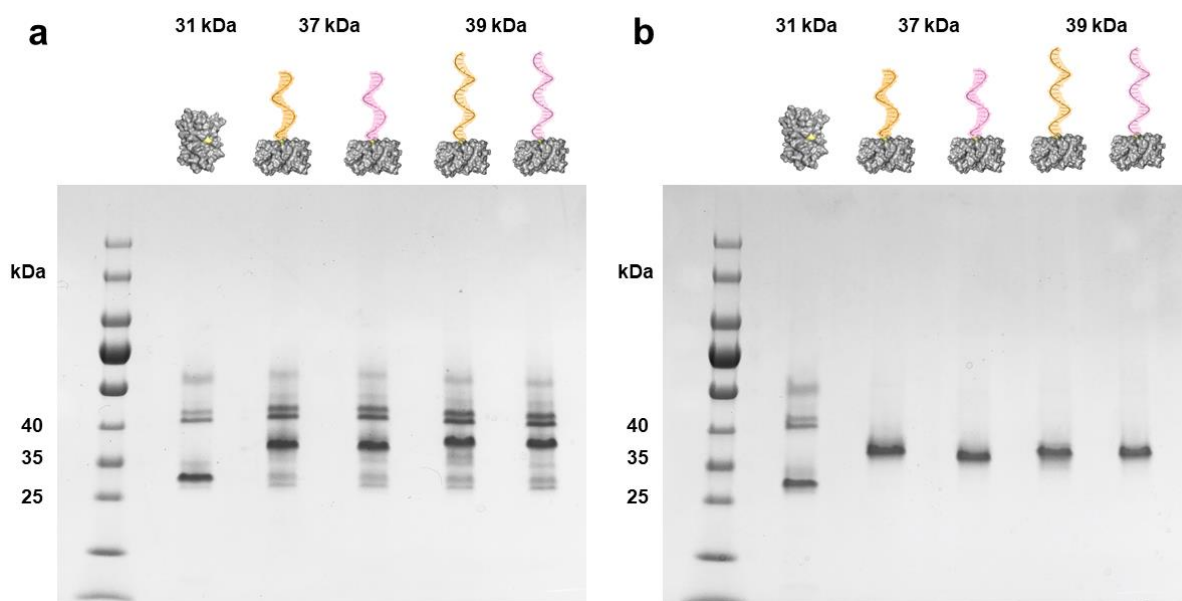
Figure S2. SDS PAGE analysis of unpurified (a) and purified (b) DNA conjugation reactions with surface cysteine. Anion exchange chromatography was employed to remove unreacted protein and expression impurities.

S3.2. Functionalization of surface lysine residues to form Janus domains

A 1.5 mL sample of 10 µM mEGFP-DNA was reacted with 1000 equivalents of NHS-PEG$_4$-N$_3$ (Thermo Scientific). The reaction between surface accessible lysine residues and NHS-PEG$_4$-N$_3$ was allowed to proceed at 25 °C for 3 h while shaking at 800 rpm on a Benchmark Multi-therm shaker. Azide labelled mEGFP-DNA was purified by size exclusion chromatography using NAP25 columns (GE Healthcare), equilibrated with PBS (pH 7.4), and the extent of reaction was quantified using MALDI-TOF (Figure S3A). Subsequently, the pendent azides were then then reacted with 150 equivalents of 5´ DBCO functionalized DNA, for 48 h at room temperature, at a protein concentration of 4.5 µM. Unreacted DNA was then removed via 10 rounds of centrifugation in 50 kDa molecular weight cut-off filters (Millipore). Janus domains A and B were further purified using size exclusion chromatography (SEC650, Bio-Rad) (Figure S4), and the fractions corresponding to the highest MW sample was collected. The DNA:Protein ratio of Janus domains was calculated, using UV-vis absorbance spectroscopy (Varian Cary 5000) (Figure S3b and c), to be 14 strands on average per protein. Native PAGE analysis of each "domain" (Figure 1e, main text) revealed several discreet bands, representative of a distribution in number of DNA strands per protein.
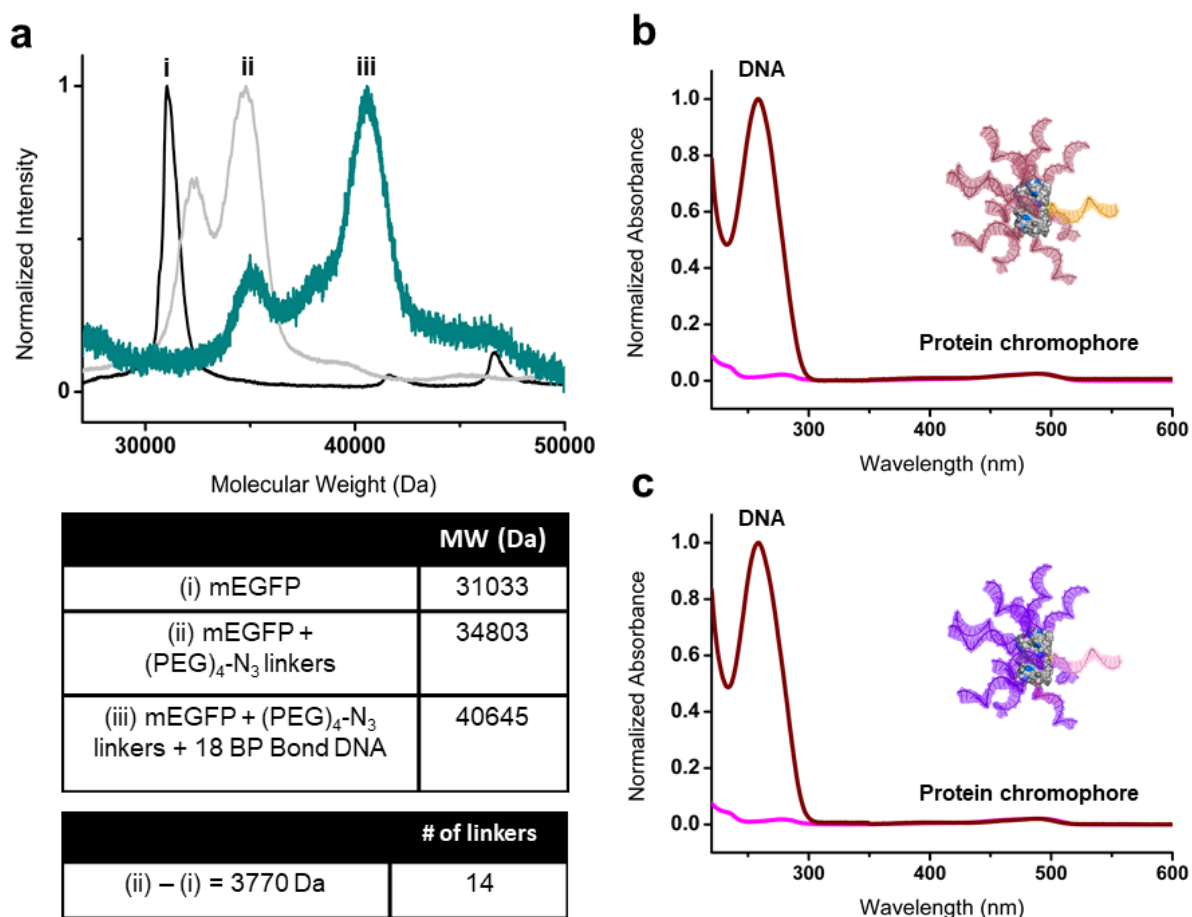
Figure S3. Mass characterization of mEGFP and intermediates using MALDI. (a) Native mEGFP in (i) sinapinic acid (SA) matrix, (ii) mEGFP after functionalization of surface lysine residues with $(PEG)_4$-N_3 linkers in SA matrix, and (iii) mEGFP + linkers and surface cysteine functionalization with DNA in 3-Hydroxypicolinic acid matrix. Table shows masses of (i-iii) obtained from MALDI. Number of $(PEG)_4$-N_3 linkers calculated using molecular weight of linker: 274 Da. (b and c) Determination of the number of DNA strands (DBCO-modified strand C (red) and D (Blue)) per protein from concentration ratios measured using UV-vis spectroscopy. Protein and DNA concentrations were calculated from the absorbance at 488 and 260 nm, respectively. All traces of a single plot are normalized to the same protein concentration.

S3.3. Assembly of Janus particle

Equimolar samples of Janus domains A and B were combined and left for 16 h to form the DNA "bond" through hybridization. The Janus particles were then purified using size exclusion chromatography (SEC650, Bio-Rad) (Figure S4), and the fractions corresponding to the highest MW sample was collected. Native PAGE confirmed the formation of the Janus particle (Figure 1e of the main text).
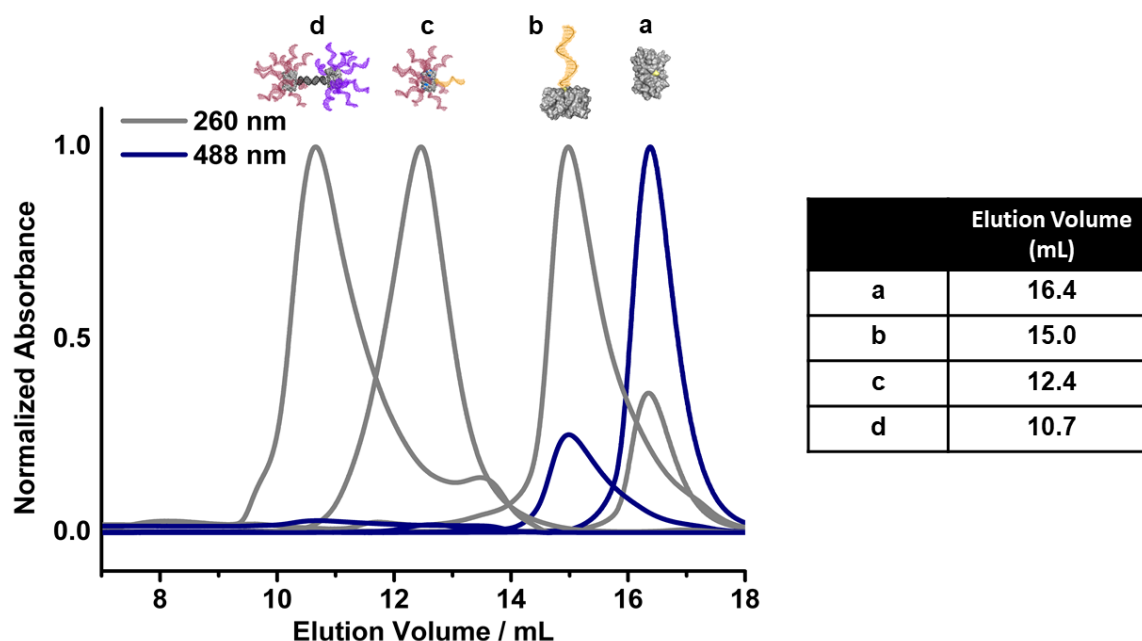
Figure S4. Size exclusion chromatography (SEC) traces of the (a) native protein, (b & c) protein-DNA conjugates, and (d) protein Janus particle. Samples were run on a Bio-Rad SEC650 column 1xPBS at 4 °C at a flow rate of 0.5 mL/min. The elution of products at decreasing elution volumes correlates with extent of functionalization. In addition, the table showing the ratios of extinction at 260:488 nm supports the successful high-density functionalization of DNA on the surface of the protein.

S4. DNA functionalization of Au & Ag nanoparticles

S4.1. Au NPs

Citrate-capped AuNPs with 10 and 5 nm diameters were obtained from Ted Pella and functionalized with 5′ thiol terminated DNA (Table S2). Firstly, disulfide (C6 SS) DNA was reduced in 100 mM dithiothreitol (DTT) (Invotrogen) for 1 h at room temperature, after which, reduced DNA was purified using a NAP25 size exclusion column (GE Healthcare). To two batches of each NP size, ~5 nmols of the two different 5′-thiolated oligonucleotide were added per mL of AuNPs and the resulting solution was incubated for 4 h at room temperature. Aliquots of 5 M NaCl were added to the solution in 0.1 M increments over the course of 3 h to reach a final concentration of 0.5 M NaCl. This solution was then allowed to incubate for 48 h, at room temperature, to maximize DNA loading on the surface of the AuNPs. The DNA-functionalized particles were purified by five rounds of centrifugation at 21,130 × g, in 100 kDa molecular weight cut-off filters (Millipore), followed by resuspension of the resulting pellet in 1 mL of 1x PBS. Particle concentrations were determined based on UV-vis absorbance

spectra (Varian Cary 5000) using molar extinction coefficients provided by Ted Pella; www.tedpella.com/ gold_html/gold-tec.htm.

S4.1. Ag NPs

Citrate-capped AgNPs with 10 nm diameters were obtained from nanoComposix and functionalized with 5´ cyclic disulfide terminated DNA (Table S2). To two batches of each NP size, ~5 nmols of the two different 5´ cyclic disulfide modified oligonucleotide were added per mL of AgNPs and the resulting solution was incubated for 24 h at 4 °C in the dark. Aliquots of 5 M NaCl were added to the solution in 0.1 M increments over the course of 3 h to reach a final concentration of 0.5 M NaCl. This solution was sonicated for 10 min after each aliquot of 5M NaCl. The final solution was incubated for 48 h, at 4 °C in the dark, to maximize DNA loading on the surface of the AgNPs. The DNA-functionalized particles were purified by five rounds of centrifugation at 21,130 × g, in 100 kDa molecular weight cut-off filters (Millipore), followed by resuspension of the resulting pellet in 1 mL of 1x PBS. Particle concentrations were determined based on UV-vis absorbance spectra (Varian Cary 5000) using reported molar extinction coefficients.[1]

S5. Crystallization and characterization of DNA-encoded protein Janus nanoparticle with Ag and Au NPs

S5.1. Colloidal crystallization conditions and procedure

Samples for SAXS and electron microscopy (EM) analysis were prepared by the co-assembly of two orthogonally functionalized inorganic nanoparticles with protein Janus nanoparticles. 50 equivalents of each Janus particle linker strand (Supplementary Table S3) were added to a 400 nM solution of Janus particles in 1xPBS and 0.5M NaCl. 200 equivalents of both inorganic NP linkers (Supplementary Table S3) were added to a solution containing 100 nM concentration of each orthogonally functionalized inorganic NP (A and B) in 1xPBS and 0.5M NaCl. The formation of aggregates was observed upon the combination of 50 μL of each solution. Samples were heated to a few degrees above their melting temperature (Supplementary Figure S5) and cooled at a rate of 0.01 °C/min to 20 °C using a ProFlex™ PCR system (Applied Biosystems). Resultant crystals were characterized by synchrotron SAXS experiments conducted at the Advanced Photon Source at Argonne National Laboratory.

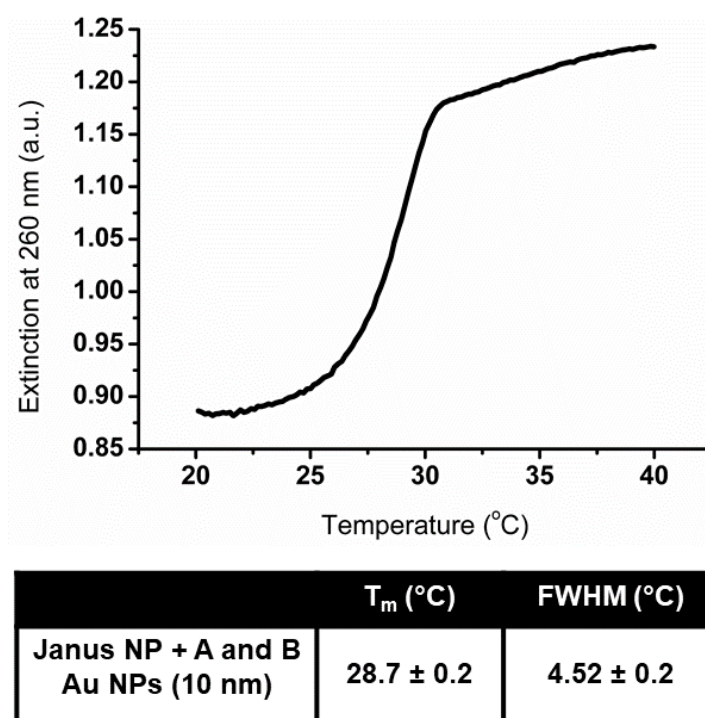|	T_m (°C)	FWHM (°C)
Janus NP + A and B Au NPs (10 nm)	28.7 ± 0.2	4.52 ± 0.2

Figure S5. Melting curve of aggregates formed from orthogonally functionalized 10 nm Au NPs and DNA encoded Janus particle (18 BP bond). Experiments were conducted using a temperature-

controlled UV-vis spectrometer (Cary5000). Solutions containing 5 nM particle and 10 nM Janus particle concentration, with 50 equivalents of both protein linkers (linkers C and D) and 200 equivalents of both AuNP linkers (linkers A and B), in 1xPBS + 0.5 M NaCl, were prepared. In order to ensure that aggregates did not form in a kinetically trapped state, samples were first heated to 40 °C and cooled to room temperature at a rate of 0.1 °C/min. Subsequently, the temperature was increased, at a rate of 0.1 °C/min, and an extinction measurement recorded at 0.1 °C intervals at 260 nm to yield the above melting curves. The melting temperature was determined by taking the maximum of the first derivative of the curve, and the full width half max (FWHM) of the first derivative is reported. Triplicate measurements were recorded to ensure reproducibility of melting character.

S5.2. Small angle X-ray scattering analysis

SAXS characterization was carried out at the DuPont-Northwestern-Dow Collaborative Access Team (DND-CAT) beamline of Argonne National Laboratory's Advanced Photon Source (APS). X-rays of wavelength 1.24 Å (10 keV) were used, and the system was calibrated using silver behenate as a standard. Two sets of slits were used to define and collimate the X-ray beam; parasitic scattering was removed via a pinhole. Slow-cooled samples were transferred into 1.5 mm quartz capillary tubes (Charles Supper) and the scattering was collected with typical exposure times varying between 0.1 and 0.5 s, depending upon the sample. The two-dimensional scattering patterns (collected on a CCD area detector) were azimuthally averaged to yield a 1-dimensional plot of intensity (I(q)) as a function of the scattering vector, q, where q is given as:

$$(1) \quad q = 4\pi sin(\theta)/\lambda$$

Here, λ is the wavelength of incident radiation and θ is half the scattering angle, 2θ. The structure factor, S(q), was obtained by dividing I(q) by the form factor of free 10 nm or 5 nm DNA functionalized inorganic NPs in 1xPBS + 0.5 M NaCl (collected at 100 nM with a 0.5 second exposure time).

S5.3. SAXS analysis and modelling of DNA mediated assembly of colloidal crystals

SAXS data consist of two basic types of scattering: form factor (P(q)), which is scattering inherent to the types of nanoparticles that are in solution, and structure factor (S(q)), which arises as a function of the arrangement of these particles relative to one another. The overall scattering of X-rays (denoted as I(q)) is a combination of these two types of scattering, and it is these values of I(q) that were measured experimentally:

$$(2) \quad I(q) = kP(q)S(q)$$

The constant, k, is a factor proportional to the square of the number of particles composing a single crystal domain, and the number of crystalline domains in the path of the X-ray beam. Since the electron density of the metal nanoparticles, particularly 10 nm AuNPs, is significantly higher with respect to other organic components in the system, the SAXS data can be used to determine, solely, the position of the nanoparticles. Colloidal crystals assembled from combinations of orthogonally functionalized (Thiol-modified oligos A and B) inorganic nanoparticles and DNA encoded protein Janus nanoparticles all adopted hexagonal lattice symmetry of P6/mmm space group. To account for differences in scattering intensity arising from inorganic nanoparticles of different composition and size, SAXS data was modeled to confirm structural assignment, as presented in Figures 3a and b of the main text. Simulated peak positions and intensities were generated using relative electron densities and sizes of Au and Ag NPs.

S5.4. Calculation and comparison of lattice parameters derived from modulation of DNA "Bond"

Lattice expansion data was obtained from the crystallization of protein Janus particles with both an 18 and 24 base-pair DNA "bond". Samples were prepared as previously stated and SAXS patterns were recorded in triplicate, from which lattice parameters were extracted (Table S4). TEM data were also collected as secondary evidence, but were not used for determination of assembly parameters, since the embedding procedure used to obtain TEM images can result in a reduction of crystal lattice parameters and decrease in crystal ordering.

Table S4. Lattice parameters measured using SAXS for three different colloidal crystal systems, assembled using Janus particles with either an 18 or 24 base-pair DNA "bond". Slow cooled samples, prepared as stated in S5.1, were measured in triplicate. Each of the three crystal systems assembled using both 18 and 24 BP Janus particles. From these data, lattice parameters were extracted and an average lattice parameter for each system was calculated.

Lattice Parameters Measured from SAXS (nm)				
1) 10 nm Au NPs (A and B) and Janus particle				
18 BP "Bond"	**Sample 1**	**Sample 2**	**Sample 3**	**Average**
a=b	37.29	37.29	37.33	37.30
c	65.04	65.18	65.18	65.13
24 BP "Bond"	**Sample 1**	**Sample 2**	**Sample 3**	**Average**
a=b	37.93	37.92	37.93	37.93

c	67.30	67.30	67.14	67.25
2) 5 nm Au NPs (A and B) and Janus particle				
18 BP "Bond"	**Sample 1**	**Sample 2**	**Sample 3**	**Average**
a=b	35.85	35.85	35.77	35.82
c	61.02	61.14	61.14	61.10
24 BP "Bond"	**Sample 1**	**Sample 2**	**Sample 3**	**Average**
a=b	36.06	36.06	36.09	36.07
c	62.86	63.24	64.14	63.41
3) 10 nm Au NP (A), 5 nm Au NPs (B) and Janus particle				
18 BP "Bond"	**Sample 1**	**Sample 2**	**Sample 3**	**Average**
a=b	36.39	36.39	36.46	36.41
c	63.51	63.38	63.51	63.47
24 BP "Bond"	**Sample 1**	**Sample 2**	**Sample 3**	**Average**
a=b	36.67	36.67	36.68	36.67
c	65.05	65.05	65.57	65.22

Table S5. The calculated difference between the average length of the lattice parameters measured for crystals containing Janus particles with either an 18 or 24 base-pair bond, across each of the three crystal systems.

	1	**2**	**3**
a=b	0.62 nm ± 0.02 nm	0.25 ± 0.07 nm	0.26 ± 0.04 nm
c	2.11 nm ± 0.15 nm	2.31 ± 0.68 nm	1.76 ± 0.30 nm

S5.5. Electron microscopy imaging of superlattices

Lattices were first embedded in silica, using a previously reported procedure,[2] to preserve their solution structure. To a suspension of slow cooled superlattice samples (100 μL) in 0.5 mL 1xPBS + 0.5 M NaCl, 2 μL of the ammonium salt, N-trimethoxysilylpropyl-N,N,N-trimethylammonium chloride (TMSPA) was added. After 15 min of gentle rotation of the Eppendorf tubes, 1.5 uL of triethyoxysilane was added. This mixture was shaken for 24 h at 600 rpm, after which it was centrifuged at 10,000 rpm (10 s) to encourage aggregates to pellet at the bottom of the tube. The supernatant was removed and replaced with water and this process was repeated 5 times to wash the silica embedded samples. Lattices were then deposited onto a carbon coated Cu-mesh TEM grid (Ted Pella). Images were obtained using a 200 keV acceleration voltage on a Hitachi HD-2300 STEM (Figure S6).
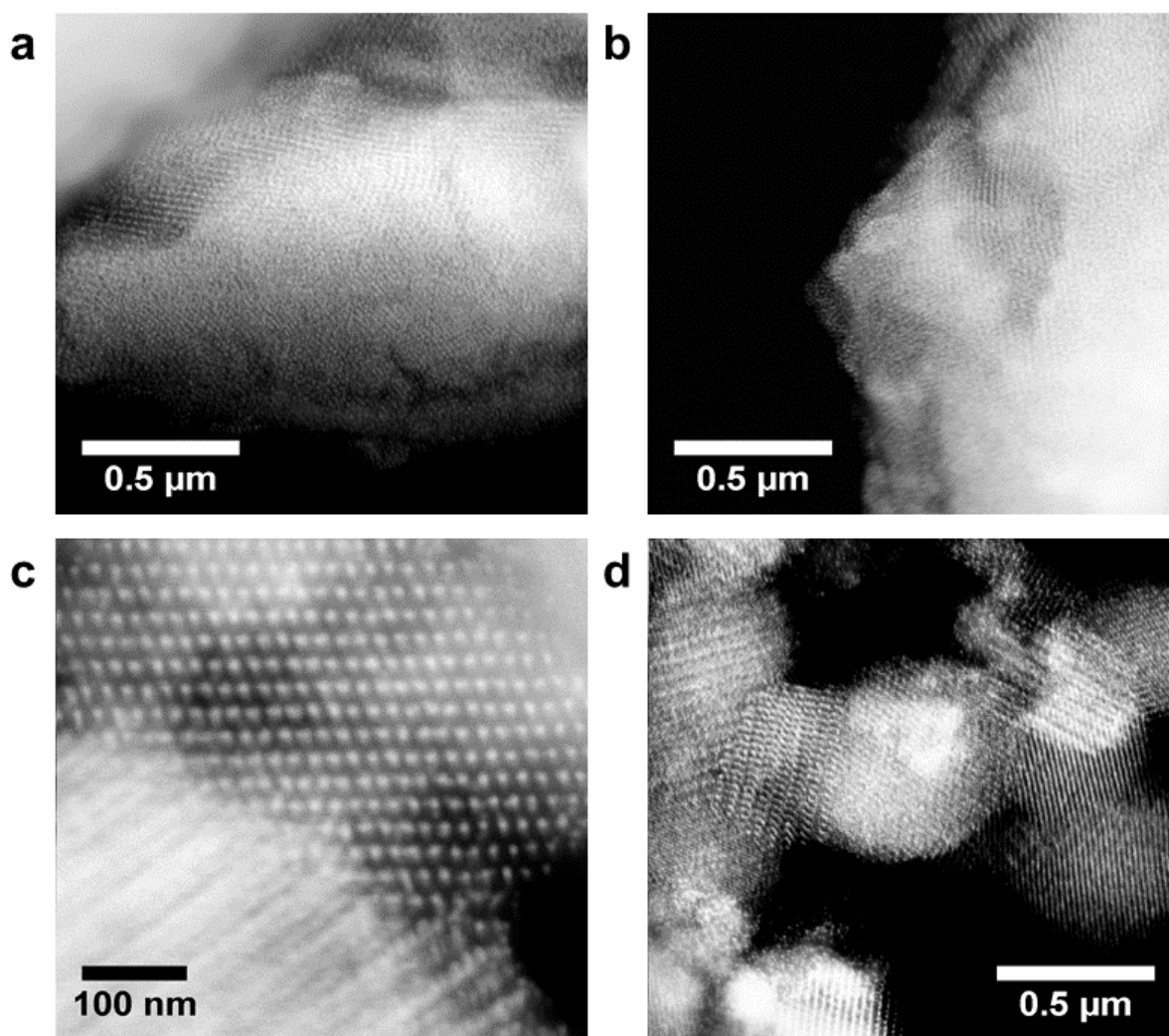
Figure S6. Representative TEM images of silica embedded superlattices, formed using DNA encoded protein Janus nanoparticles. (**a**) and (**b**) depict long range ordering of two orthogonally functionalized 10 nm Au NPs, arising from the DNA mediated interactions with protein Janus particles. In (**c**) and (**d**) a crystal assembled from orthogonally functionalized 5 and 10 nm AuNPs is visualized using STEM, from which we observe both the hexagonally packed and layered planes, characteristic of the AlB_2 structure type.

Samples of slow cooled, silica embedded superlattices were enrobed in 2% low melt Agar in water. The agar, containing superlattice, was extruded into small tubes using a glass pipette tip. These tubes were then transferred to 2 mL of 30% ethanol in water, followed by slow replacement of the solvent to obtain samples in pure ethanol. Next, the solvent was slowly replaced by pure propylene oxide, and once again by resin (Embed 812, Electron Microscopy Sciences); the slow transfer process ensured that the resin fully infiltrated the sample prior to solidification. Samples were heated at 65 °C for 48 h to solidify the resin. The resin encased superlattices were sectioned into ~60 nm thick slices that could be imaged using a 200 keV acceleration voltage on a Hitachi HD-2300 STEM (Figure S7).
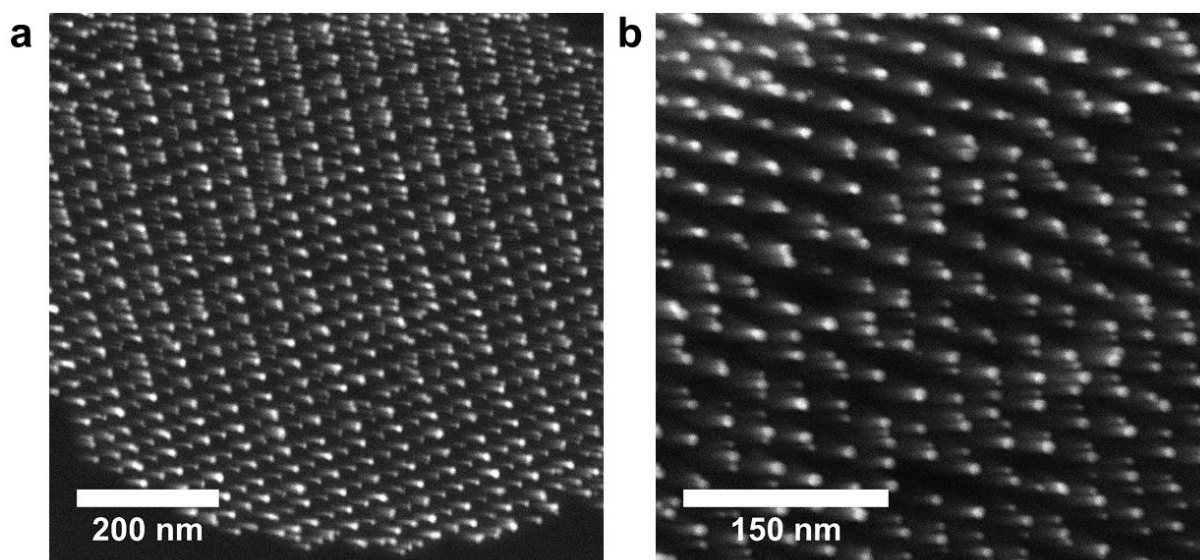
Figure S7. Representative TEM images of a sectioned (~60 nm) crystal system containing orthogonally functionalized 5 and 10 nm Au NPs. **(a)** reveals the layers of 5 and 10 nm Au NPs, **(b)** shows the hexagonally packed planes.

S5.6. Supplementary discussion regarding AB$_2$ lattice

A key design rule regarding DNA-mediated nanoparticle crystallization states that particles will form a lattice such that they maximize the number of nearest neighbours to which they can form DNA connections.[3,4,5] Additionally, any non-complementary DNA interactions may be considered repulsive, and therefore energetically unfavourable. Upon consideration of the symmetry of the Janus particle's DNA functionalization, a perpendicular alignment of the Janus particle with respect to hexagonally ordered planes of orthogonally functionalized AuNPs would maximize the number of interactions between a given domain and its respective complementary set of AuNPs. Based on the symmetry of the Janus particle and the thermodynamic driving force to maximised hybridization events, the possible location and orientation of the protein Janus particle within the unit cell is restricted. The Janus particle must adopt a vertical orientation thus supporting the hypothesis of forming an AB$_2$ lattice since two Janus particles are required to maximize interactions between nanoparticles occupying (0,0,0) and (0,0,1/2) positions.

References

1.   Paramelle, D.; Sadovoy, A.; Gorelik, S.; Free, P.; Hobley, J.; Fernig, D. G., *Analyst* **2014,** *139,* 4855-4861.

2.   Auyeung, E.; Macfarlane, R. J.; Choi, C. H. J.; Cutler, J. I.; Mirkin, C. A., *Adv. Mater.* **2012,** *24,* 5181-5186.

3.   Macfarlane, R. J.; Lee, B.; Jones, M. R.; Harris, N.; Schatz, G. C.; Mirkin, C. A., *Science* **2011,** *334,* 204-208.

4.   Macfarlane, R. J.; O'Brien, M. N.; Petrosko, S. H.; Mirkin, C. A., *Angew. Chem. Int. Ed.* **2013,** *52,* 5688-5698.

5.   Seo, S. E.; Li, T.; Senesi, A. J.; Mirkin, C. A.; Lee, B., *J. Am. Chem. Soc.* **2017,** *139,* 16528-16535.