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Screening of Combinatorial Peptide Libraries for Nanocluster Synthesis

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Summary

A significant challenge in bionanotechnology is the discovery of effective biological interfaces that allow inorganic nanoscale materials to mimic effectively their biological counterparts. Much like *de novo* design of proteins, the rational design of such interfaces is a daunting task. An alternative approach is to screen libraries of peptides, inspired by known biological examples of such hybrid protein–material interfaces, for peptide ligands capable of not only stabilizing a size-discrete population of nanoclusters, but providing the requisite biological compatibility. The protocol described in this chapter is an approach for the simultaneous screening of spatially addressable combinatorial libraries for the stabilization of a variety of metal sulfide, metal oxide, and zero-valent nanoclusters. Additionally, the screening process allows the researcher to characterize the resulting nanoclusters in terms of a variety of physical properties. Ultimately, an informatics structure–function analysis may be performed in order to elucidate specific properties of the ligand sets, which provides access to certain desired material characteristics.

Key Words

Combinatorial chemistry; nanocluster synthesis; biomimetic nanoclusters; peptide-encapsulated nanoclusters; spatially addressable combinatorial library; metal sulfide nanoclusters; zero-valent nanoclusters.

1. Introduction

A major objective in achieving robust, functional nanodevices is the development of the requisite chemistry to assemble larger architectures through control of the interfaces and distribution of nanocomponents. Biological systems offer important insights into possible approaches to the problems encountered in the synthesis of extended materials ***(1)***. The array of materials produced by biological systems include laminate composites and ceramics such as bone, teeth, and shells ***(2)***; magnetic materials such as the forms of magnetite found

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in magnetobacteria and the brains of migratory animals *(3)*; novel silver or cadmium sulfide nanoclusters produced as a result of heavy metal detoxification mechanisms by bacteria *(4,5)*; and arrays of precisely fabricated diffracting architectures resulting in the multitude of intense colors observed in insects and birds *(6)*. Consequently, there has been increasing interest in the use of biomolecules to control the synthesis of nanoparticles in a biomimetic fashion.

The relationship of ligand structure to chemical and physical properties of the resulting cluster is a central theme in nanoscale chemistry. Advances in a number of areas, such as catalysis and sensor discovery, highlight the utility of complexes with well-designed structural, electronic, and stereochemical features. Unfortunately, the rational design of ligands for functional nanoclusters remains extremely empirical, especially if novel physical and chemical properties are desired. Increasingly, combinatorial chemistry is emerging as a viable approach for the identification of such novel ligands (**Fig. 1**) *(7)*. In this chapter, we present our approach for screening peptides *(8,9)*, derived from a spatially addressed peptide library, for the ability to stabilize novel biologically compatible and functional nanoscale materials.

2. Materials

2.1. Nanocluster Synthesis

1. Peptide libraries: There are three primary approaches to the development of synthetic ligand libraries *(10)*. Conventional serial approaches result in "one-at-a-time ligands." Although this method provides a high level of control over the compounds entering the screening process, it results in a low throughput of potential compounds. Pooled synthesis combinatorial approaches represent the other end of the spectrum. Methods such as the "split-pool" approach yield an extremely large number of compounds but present challenges of purity and deconvolution of assay screens. An intermediate approach is the parallel synthesis of an array of compounds in a spatially addressable format. For the purpose of this protocol, the library used is a spatially addressable library that has been synthesized using the Mimotopes peptide on a pen system *(11)*. Peptide synthesis yielded approx 1–4 µmol of each target ligand. Control peptides were >97% pure by high-performance liquid chromatography and mass spectrometry. It should be emphasized that any well-designed library will suffice as the basis of the assay screen. Subsequently, the individual members of the peptide library were screened simultaneously for the ability to stabilize a wide spectrum of nanoparticles, followed by screens to evaluate the physical properties of the resulting materials. Lyophilized peptides were dissolved in 1.0 mL of 0.1% trifluoroacteic acid (TFA), resulting in a final peptide stock solution with a concentration of 1.0–4.0 m*M*.
2. Aqueous solvents: Prepare 0.01 *M* HCl, 0.1% TFA, and double-deionized water filtered by reverse osmosis and ion-exchange cartridges (MODULAB water

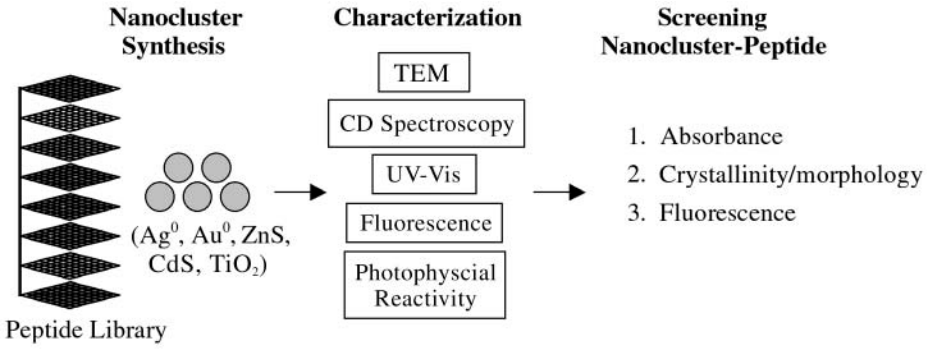

Fig. 1. Total possible number of permutations varied over three binding positions and eight amino acids to comprise peptide library.

systems) in 500-mL total volumes. Store the 0.1% TFA in a glass bottle. After solvent preparation, degas each solvent for 30 min with N_2 on a Schlenk line (*see* **Note 1**). Then cycle the solvents into a glove box and store.

3. 0.01 *M* Phosphate buffer, pH 6.0 (*see* **Note 2**): Dissolve 0.1648 g of sodium phosphate dibasic Na_2HPO_4 and 0.6050 g of sodium phosphate monobasic NaH_2PO_4 in 500 mL of double-deionized water. Adjust the pH to 6.0 with 1 or 2 drops of 1 *M* HCl. Degas the buffer with N_2 for 30 min prior to storage in a glove box.
4. Stock solutions of metal ions at a concentration of 5 m*M*: In four 10-mL glass vials, weigh 0.00718 g of $ZnSO_4$, 0.00521 g of $CdSO_4$, 0.00425 g of $AgNO_3$, and 0.00984 g of $HAuCl_4 \bullet 3H_2O$. Cycle into a glove box through the antechamber (*see* **Note 3**). In the glove box, dissolve $ZnSO_4$ and $CdSO_4$ in 5.00 mL of 0.01 *M* HCl. Dissolve $AgNO_3$ and $HAuCl_4 \bullet 3H_2O$ in 5.00 mL of double-deionized water. Store the $HAuCl_4 \bullet 3H_2O$ covered with aluminum foil (light sensitive) in a refrigerator at 4°C. Additionally, cover the $AgNO_3$ solution with aluminum foil (light sensitive).
5. Titanium (IV) isopropoxide ($Ti([CH_3]_2CHO)_4$) solution: Pipet 7.3 µL of $Ti([CH_3]_2CHO)_4$ in 5.00 mL of absolute ethanol in a separate vial to yield a 5 m*M* solution under nitrogen (*see* **Note 4**). Store under N_2 in a glove box to avoid all humidity. $Ti([CH_3]_2CHO)_4$ is readily hydrolyzed and will instantly precipitate as TiO_2 in even the slightest levels of humidity; it is extremely sensitive to moisture. Make solution daily as needed.
6. 5 m*M* Stock solution of inorganic sulfide: Dissolve 0.00195 g of Na_2S in 5.00 mL of double-deionized water in a glove box by first weighing out the appropriate amount of Na_2S in a glass vial and then transferring to the glove box. Keep refrigerated and prepare once a week.
7. 5 m*M* Stock solution of sodium borohydride reductant: Dissolve 0.00189 g of $NaBH_4$ in 10.00 mL of double-deionized water under nitrogen. Prepare daily; $NaBH_4$ loses reducing power over time.

2.2. Screening of Library Peptides

1. Basic ultraviolet-visible (UV-Vis) spectrophotometer, either scanning or equipped with a photodiode array. To increase throughput, an Agilent 8453 spectrophotometer model with a multicell holder can be employed.
2. Varian Cary Eclipse fluorometer, to screen the set of metal sulfide nanoclusters (ZnS and CdS) for fluorescence.
3. Aviv model 215 CD spectrometer equipped with a thermoelectric cell holder accessory, to perform circular dichroism (CD) spectroscopy.
4. Phillips CM20 transmission electron microscope operating at 200 kV and coupled with an energy-dispersive X-ray spectrometer, to provide physical characterization of the nanoclusters.
5. Methylviologen dye, to examine the semiconducting nanoclusters of ZnS, CdS, and TiO_2 for photophysical reactivity. Prepare a 12.5 m*M* methylviologen solution by pipetting 12.5 µL of a 0.01 *M* methylviologen solution with 1 mL of 0.1 *M* NaOH in a 10-mL volumetric flask and diluting with double-deionized water. Cover the methylviologen solution with aluminum foil (sensitive to light) and store in a glove box.

3. Methods

3.1. Nanocluster Synthesis With Library Peptides

Each nanocluster type (Au^0, Ag^0, ZnS, CdS, and TiO_2) is synthesized in triplicate to yield a total of 15 reactions carried out for each peptide (*see* **Note 5**). All nanocluster reactions are performed in the anaerobic environment of a glove box according to the general reaction scheme in **Fig. 2**.

3.1.1. Reductive Synthesis of Ag^0 and Au^0 Nanoclusters

1. Add 1.00 mL of 10 m*M* phosphate buffer, pH 6.0, to six 10-mL glass vials.
2. To each vial, pipet 10.0 µL of the 3.7 m*M* peptide solution.
3. To a set of three labeled vials, add 4.8 µL of $AgNO_3$ stock solution. To the remaining set, add 4.8 µL of $HAuCl_4 \cdot 3H_2O$ stock solution (*see* **Note 6**).
4. Add a micro stir bar to each vial.
5. Wait 15 min after adding metal ions to the peptide to allow for the formation of a metal-peptide precursor complex.
6. Pipet 9.6 µL of 5 m*M* sodium borohydride to all vials to initiate reduction.
7. Cover the vials in aluminum foil and stir for 4 h.

3.1.2. Sulfide Addition Synthesis of ZnS and CdS Nanoclusters

1. To six vials, add 1.00 mL of 10 m*M* phosphate buffer, pH 6.0.
2. Add 10.0 µL of a library peptide to all vials.
3. To a set of three vials, pipet 4.8 µL of the 5 m*M* stock solutions of $ZnSO_4$ or $CdSO_4$.

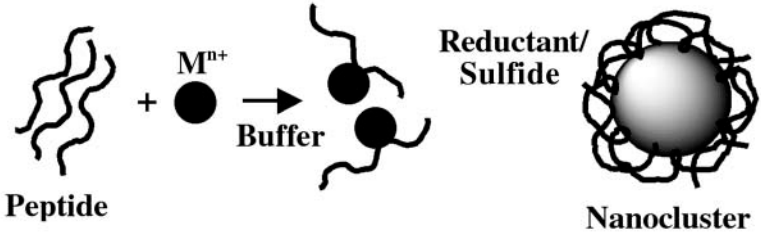

Fig. 2. Nanocluster synthesis scheme with peptide library.

4. Add a micro stir bar to each vial.
5. After 15 min, add 4.8 µL of 5 m*M* Na_2S to all six vials to yield a final peptide concentration of 3.6289×10^{-5} *M*.
6. Cover the vials with aluminum foil and stir for 4 h.

3.1.3. Condensation Synthesis of TiO_2 Nanoclusters

1. To the last set of three labeled vials, add 1.00 mL of 10 m*M* phosphate buffer, pH 6.0.
2. Pipet 10.0 µL of peptide into each vial.
3. Dispense 4.8 µL of 5 m*M* stock solution of $Ti([CH_3]_2CHO)_4$ to each peptide-containing vial.
4. Add a micro stir bar, cover with aluminum foil, and stir for 4 h.

3.2. Screening Library

3.2.1. Secondary Structure of Peptide Ligand

During the 4-h nanocluster reaction period, perform the following preliminary studies on the free peptide: individual CD spectrum and temperature unfolding study.

1. Pipet 5.0 µL of peptide in 0.5 mL of 10 m*M* phosphate buffer, pH 6.0. Transfer the peptide solution to a strain-free CD quartz cell with a 1-cm path length.
2. Examine the peptide for a secondary structure using a CD spectrometer to scan the sample from 190 to 260 nm with 0.5-nm steps averaging 2 s at every step to obtain the CD spectra of the free peptides. After spectra are collected, convert all raw data to molar ellipticity (units of degrees•cm^2/dmol) by dividing by the concentration and the path length of the cell.
3. If there is a secondary structure of interest, obtain a melting profile of the free peptide over the temperature range of 5 to 85°C at 10°C intervals. Most commercial CD instruments have an appropriate programmable interface. Use this to program the thermal denaturation experiment beginning at 5°C using a 15-min temperature equilibration period at each new temperature (*see* **Note 7**).

3.2.2. Characterization of Peptide-Encapsulated Nanoclusters

On completion of synthesis, perform a methylviologen reduction assay while the reactions are under nitrogen. Perform this assay on the CdS, ZnS, and TiO_2 nanoclusters.

1. In a glove box, mix 100 µL of the crude nanocluster reaction with 800 µL of the 12.5 m*M* methylviologen basic solution in a 1.00-mL cuvet with a rubber stopper. Put the cuvet in a foil-covered beaker and remove from the glove box. Obtain a UV-Vis spectrum of the solution and identify λ_{max}. Using a fluorometer, excite the sample at λ_{max} for 10 min without acquiring data (set the excitation monochromator and scan parameters, but do not start the scan). After irradiation, carefully remove the sample from the fluorometer and place back in the foil-covered beaker. Take a UV-Vis spectrum of the sample against an air blank (*see* **Note 13**). The appearance of peaks in the spectrum at 398 and 600 nm indicates a positive result for the photodriven reduction of methylviologen.
2. Remove all 15 reaction vials from the glove box and obtain UV-Vis absorbance spectra using a 1.00-mL cuvet (*see* **Note 8**).
3. Additionally, obtain CD spectra for all nanocluster reactions following above parameters (*see* **Note 9**).
4. If there is a secondary structure of interest on the nanoparticle surface, select the most representative sample that exhibits reproducibility from the triplicate CD spectra and obtain a CD melting profile from 5 to 85°C using a temperature macro (**Fig. 3**). Repeat the temperature experiment with all five nanoclusters (*see* **Note 10**).
5. Examine the clusters of CdS and ZnS for fluorescence. Perform an emission scan on nanoclusters by exciting samples at the absorbance maximum determined in the UV-Vis spectrum. Start the scan range at 10 nm above the excitation wavelength and stop the scan at 750 nm. As needed, adjust the photomultiplier tube detector voltage to the highest value, and set the excitation and emission slits to 5 nm (*see* **Note 11**).
6. Study the physical characteristics of the nanoclusters by transmission electron microscopy (TEM). Prepare TEM grids (200-mesh quantifoil copper grids from SPI supplies) by pipetting 2 drops of the crude aqueous nanocluster reaction onto a grid for each different nanocluster (*see* **Note 13**). Obtain a size distribution histogram from the TEM micrograph by manually measuring 50–100 particles in the bright-field image (**Fig. 4**).

3.2.3. Evaluation Criteria for Nanocluster-Peptide Screens

Inspect the morphology and crystallinity of nanocluster populations in the TEM micrographs for uniformity and appearance. Nanoclusters should appear spheroidal or faceted. An absence of these physical features is indicative of ineffectual stabilization of nanocluster, and the reaction would be considered unsuccessful.

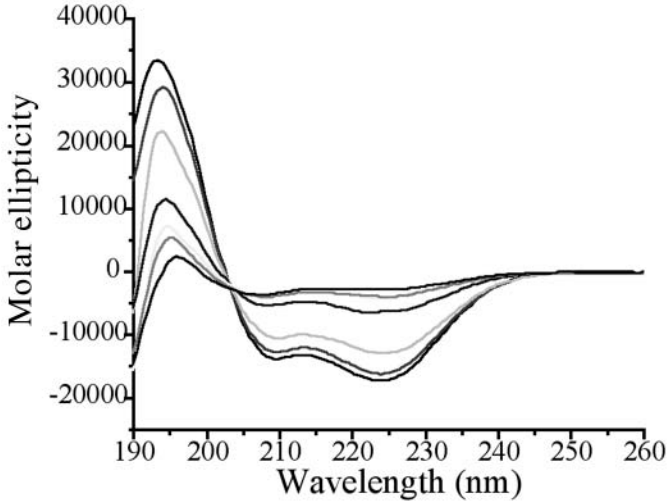

Fig. 3. CD spectra of ZnS-(HHH) obtained over temperature range of 25–85°C.

The CdS and ZnS nanoclusters should fluoresce near 530 and 440 nm, respectively, as broad peaks. Lack of fluorescence suggests poor stabilization of nanoparticles by screened ligand.

Each type of nanocluster should exhibit characteristic absorbance maxima λ_{max} (*see* **Note 8**). Lack of the characteristic quantum confined absorbance for semiconductor nanoclusters or plasmon resonance absorption features for noble metal nanoclusters is indicative of an unsuccessful reaction.

After identifying the peptide-nanocluster "hits," reaction conditions may be optimized for specific peptide-encapsulated nanocluster combinations.

4. Notes

1. Solvents can also be purged using an HPLC buffer purge system with helium for 30 min or frozen/thawed under vacuum to remove dissolved gases.
2. Phosphate buffer provides the pH desired as well as less signal to noise in CD spectra. Consequently, the peptides exhibit maximal helicity and the effects of the nanocluster surface are obscured in more basic pH, so pH 6.0 was chosen.
3. Use a Kimwipe and rubber band to cap the vials, preventing oxygen from being trapped.
4. Make sure the pipet tip is in the ethanol before dispensing; otherwise, TiO_2 is likely to form.
5. Nanocluster reactions are done in triplicate to ensure reproducibility. It should be emphasized that the nanocluster reaction conditions represent an averaged set of conditions expected to work for the widest possible variety of nanoclusters screened. This does not imply that the reaction conditions for nanocluster formation are

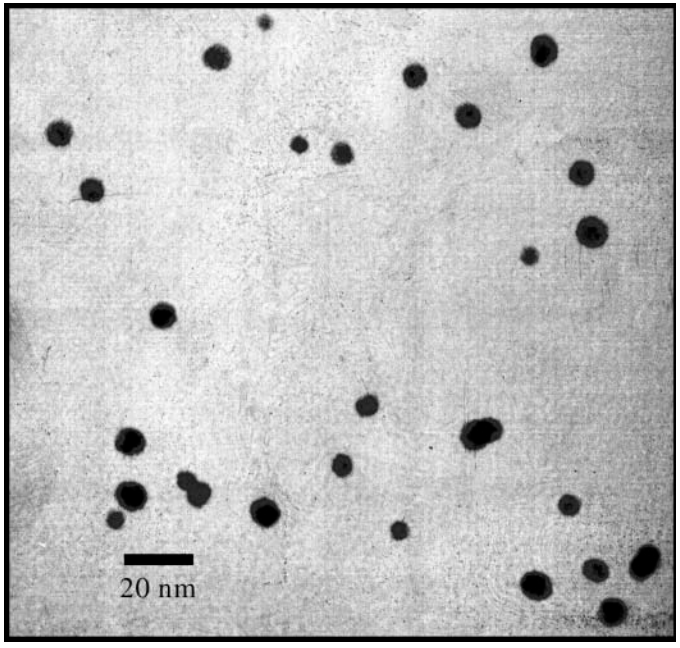

Fig. 4. Bright-field TEM micrograph of representative sample of Au^0-(HAA) nanoparticles in 10 m*M* phosphate buffer, ph 6.0, and at magnification of ×310,000.

necessarily optimized. Once "hits" have been identified, the specifics of the reaction space for any nanocluster/peptide combination should be systematically optimized.

6. The addition of metal and peptide is in a 1 : 1.25 mole ratio.
7. Thermal denaturation experiments take approx 3.5 h.
8. When transferring cuvets from the fluorometer to the UV-Vis spectrophotometer, do not disturb the sample by mixing or shaking. Any convection of sample will cause the assay to fail, because the trapped holes on the nanocluster surface will recombine with the reduced methylviologen and oxidize the dye, resulting in loss of characteristic absorption features.
9. Examine UV-Vis spectra for the characteristic absorbance of each cluster type. The zero-valent metal nanoclusters have strong plasmon absorption benchmarks indicative of formation at about 400–460 nm for Ag^0 and 500–550 nm for Au^0. The metal sulfides exhibit λ_{max} at about 260–300 nm less than their bulk band-gap energy.
10. Superimpose CD spectra from the reactions performed in triplicate to confirm reproducibility.
11. Plot temperature data by measuring the molar ellipticity at 222 nm for each subsequent temperature; this yields a plot of temperature vs helicity.

12. Depending on the fluorometer model available, scan parameters such as slit widths, detector voltages, and scan averaging times may need to be optimized.
13. After approx 30 s of immersing the grid, remove the grid from the droplets and air-dry for several minutes. There are a number of techniques for preparing samples, but this allows enough of the sample to deposit onto the grid for observation.

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