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Peptide-encapsulated CdS nanoclusters from a combinatorial ligand library†

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A spatially addressable combinatorial library of peptides, based on cysteine containing phytochelatins, has been shown to stabilize a variety of discrete CdS nanoclusters ranging in size from 19 to 26 Å in diameter.

Quantum confined structures have elicited a great deal of recent interest.[1] An important class of these compounds is the II–VI semiconductor nanoclusters exemplified by CdS. Although there are a variety of excellent techniques for the synthesis of these clusters, the use of thiolate ligands to cap the growing CdS cluster *in situ* yields nanoclusters more resistant to aggregation and more readily processable.[1e] Interestingly, a variety of yeast, plants and bacteria use a class of cysteine containing peptides known as phytochelatins to trap nearly monodisperse CdS nanoclusters, thereby efficiently sequestering a large number of cadmium ions per peptide.[2] The sequence of the phytochelatins is generally $(\gamma\text{-GluCys})_n\text{Gly}$, where *n* is the number of dipeptide repeats.[3] Comparisons between the $(\gamma\text{-GluCys})_2\text{Gly}$ peptide and the more common α-peptide linkage show that $(\alpha\text{-GluCys})_2\text{Gly}$ stabilizes smaller nanoclusters with higher band gap transitions than those with γ-linkages.[4] To investigate the influence of thiolate separation in multidentate capping peptides on the stabilization of various populations of CdS nanoclusters, a spatially addressable combinatorial library in which the spacer amino acids have been systematically varied for the n_3 phytochelatin, X-Cys-Y-Cys-Z-Cys-Gly, has been synthesized. We report, herein, the analysis of those peptides that lie along the library's diagonal in which X, Y, and Z are equivalent amino acids.

Based on the n_3 phytochelatin sequence X-Cys-Y-Cys-Z-Cys-Gly, the cysteine residues are kept constant, while the spacer residues are varied over all combinations of the spacer amino acids, resulting in an initial library of 125 peptide ligands. The five selected spacer residues include α-Glu, γ-Glu, γ-aminobutyric acid (GABA), SerGly and ε-aminohexanoic acid (ε-Ahx). The spacer residues include natural and non-natural amino acids having between 3 to 7 atoms in the backbone and spanning an idealized extended C_α–C_α distance between the cysteines ranging from 3.75 Å to 8.7 Å, respectively.[5] Additionally, the spacer residues provide a wide range of hydrophilicity. The library was synthesized using Chiron Technologies PepSets system.[6] Peptide synthesis yielded approximately 1 mmol of each target ligand. Control peptides were greater than 97% pure by HPLC and mass spectrometry (see Electronic Supplementary Information).

The peptides were reacted anaerobically with Cd^{2+} and S^{2-} in aqueous Tris buffer (100 mM, pH 8.6) in a 1.25 : 1 : 1 ratio, respectively, for 4 h.‡ Since all of the ligands in the library are likely to stabilize CdS cluster formation, it is important that multiple screens be applied to the reaction mixtures in order to develop meaningful selection criteria for a specific nanocluster characteristic. The resulting reaction mixtures were subjected to an array of screens to assess size, monodispersity, photooptical properties, photocatalytic potential and stability. The selected screens encompass many of the most commonly cited critical parameters for this class of compounds.[1] It should be emphasized that the employed screens are designed to maximize the information about any given peptide–cluster aggregation reaction, not to definitively characterize the reaction product. The results from the analysis of the library's diagonal are given in Table 1.

Table 1 Results of combinatorial library screens

Peptide[a]	$\lambda_{abs,\ max/nm}$	R_{est}[d]/Å	$\lambda_{em,\ max/nm}$	MV^{2+} assay	PNP oxidation
ECG[b]	365	13.02	548	+	+
ECECECG	306	10.43	400	+	—
eCeCeCG	320	10.97	408	+	—
gGCgCgCG	308	10.51	419	+	—
SGCSGCSGCG	302	10.29	422	+	—
hChChCG[c]	284	9.65	423	+	—
	318	10.89	426		

[a] e, γ-Glu; E, α-Glu; g, GABA; h, ε-Ahx; S, Ser; G, Gly. [b] These results are consistent with those reported in refs. 2, 7 and 8. [c] After 24 h reaction time λ_{max} was 338 nm. R_{est} was 11.7 Å. All other reactions were unchanged after 24 h. [d] Ref 11.

The UV-visible absorption spectra of the resulting nanoclusters are shown in Fig. 1. The results indicate that each of the examined peptides is capable of stabilizing discrete populations of nanoclusters. The observed λ_{max} for the absorption spectra of the encapsulated $(\alpha\text{-GluCys})_3\text{Gly/CdS}$ (**1**) and $(\gamma\text{-GluCys})_3\text{Gly/CdS}$ (**2**) clusters at 306 and 320 nm, respectively, correspond well with previous reports for the *in vitro* synthesis of CdS nanoclusters using these peptides obtained from solid-phase peptide synthesis techniques or isolated from biological sources.[3] The absorption spectra for $(\text{GABACys})_3\text{Gly/CdS}$ (**3**) and $(\text{SerGlyCys})_3\text{Gly/CdS}$ (**4**) reveal similar distributions of clusters with λ_{max} near 313 nm. The absorption spectrum of the CdS clusters encapsulated by $(\varepsilon\text{-AhxCys})_3\text{Gly}$ (**5**) show two distinct populations of clusters centered with λ_{max} of 284 and 318 nm. Examination of **5** after 24 h shows a marked decrease in the cluster populations with λ_{max} centered near 282 and a new major population with λ_{max} centered at 338 nm. These results suggest that the cluster populations observed for the capping ligand $(\varepsilon\text{-AhxCys})_3\text{Gly}$ at 4 h represent intermediate metastable clusters which over time aggregate into the final cluster.

The photophysical properties of synthetic semiconductor nanoclusters are dictated by surface-mediated phenomena.[1a,b] Ultraviolet irradiation of the individual reaction mixtures at the respective absorption maxima resulted in luminescence in the visible spectral region with emission maxima ranging from 410 to 460 nm (Electronic Supplementary Information). The fluorescence behavior of clusters **1** and **2** is in good agreement with previously reported values.[3] Anaerobic excitation of all of the peptide encapsulated CdS nanoclusters in the presence of methyl viologen resulted in the reduction of the dye as monitored by characteristic absorbances near 380 and 600 nm. A significant back-reaction attributable to reoxidation of the dye by trapped holes (h+) on the particle's surface was also observed (Electronic Supplementary Information). In contrast to reports of glutathione (γ-GluCysGly) stabilized CdS nano-

† Electronic supplementary information (ESI): Schematic representation of the spatially addressable combinatorial library, HPLC, mass and fluorescence spectra, MV^{2+} assay, chromatograms, M_w calibration curve, model of CdS–peptide cluster. See http://www.rsc.org/suppdata/cc/a9/a907178d/

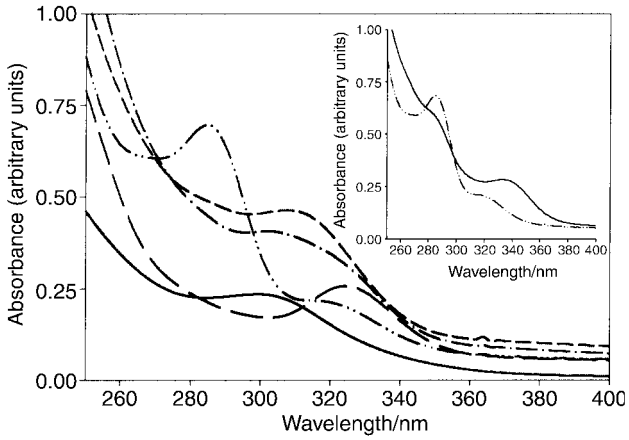

Fig. 1 UV-vis absorption spectra of the peptide–CdS cluster reaction after 4 hours. ——— **1**, — — — **2**, –·–· **3**, – – – – **4**, –···– **5**. Insert shows –···– **5** after 4 hours and ——— **5** after 24 hours.

clusters,[7,8] none of the examined nanoclusters were competent photocatalysts for the oxidative degradation of *p*-nitrophenol (PNP).

Each reaction mixture was examined for dispersity by size exclusion chromatography (Electronic Supplementary Information).[9] Chromatographic analysis of **1** and **2** revealed one major nanocluster containing fraction each, with an absorption maxima and full width at half maximum (FWHM) of the absorption peak essentially unchanged from that of the reaction mixture. Chromatograms of **3** and **4** had one major nanocluster fraction each, with a significant red shift in both the absorption maxima by 4 and 13 nm, respectively, and a 10–17% change in the absorption peak's FWHM. The resulting red shift is indicative of the separation of some fraction of smaller CdS clusters. The chromatogram of **5** resolved two major nanocluster fractions, the first containing only clusters with λ_{max} of 284 nm, while the second peak was a mixture of clusters with λ_{max} at 284 and 318 nm. The ratio of the two cluster populations is significantly altered from the crude reaction mixture demonstrating that two clusters are in fact being resolved. The chromatographic analysis clearly reveals that increasing the degree of rotational freedom within the spacer residue of the trapping ligand decreases the monodispersity of the resulting nanoclusters at the arbitrarily fixed end-point of the assay.

To verify the size of the resulting peptide-encapsulated CdS nanoclusters, a simplified compositional model of **1** and **2** was constructed using the methods of Dameron and Dance.[10] The model for the CdS core assumes an approximately equidimensional fragment of the cubic (sphalerite) lattice.§ The sizes of the CdS cores were estimated from the UV-vis spectra using Brus' effective mass model.[11] With a limiting cluster size, the number of peptides coating the particle was extrapolated from an analysis of S atom coordination. Based upon these models, the combined molecular weight of the peptide coat and CdS core was determined to be approximately 28 000 Da for nanoclusters **1** and **2**. The observed molecular weights from size-exclusion chromatographic analysis for **1** (30 137 + 5% Da) and **2** (28 438 + 5% Da) are in excellent agreement with this simplified compositional model. Similar modeling of clusters **3**–**5** is currently underway.

Through the use of a spatially addressable combinatorial library of peptide ligands inspired by the known phytochelatin sequence, peptide-coated CdS quantum-confined clusters have been synthesized. Analysis of the library's diagonal shows that the cluster size increases with the number of spacer bonds between the cysteine residues from 19.2 Å diameter for the peptide chelate $(\alpha\text{-GluCys})_3$Gly to 23.4 Å diameter for $(\varepsilon\text{-AhxCys})_3$Gly, resulting in a series of robust, photo-reductive clusters. The mechanism of size stabilizing selectivity is probably mediated by those peptides and/or peptide–$(Cd^{2+})_n$ complexes that trap growing nanoclusters when the surface of the aggregate presents sites which match the conformational spacing of the peptide or peptide complex. Such a mechanism, analogous to those proposed for the *in vivo* response of CdS sequestering organisms,[2] has important ramifications in the ability to control aggregation. From the perspective of the trapping moiety, it predicts that those peptides with greater degrees of rotational freedom in the peptide backbone may trap a variety of intermediate cluster sizes that are metastable due to steric crowding of the surface peptide coat and non-optimal surface coordination. From the perspective of the growing cluster, a trapping mechanism leads to stabilization of intermediate clusters with coordinatively unsaturated Cd^{2+} or S^{2-} ions that are shielded by the packing of the surface peptide coat. Such intermediate clusters eventually aggregate to more optimal sizes and surface coordination. This trapping termination of the growing aggregate is consistent with the intermediate clusters observed in the formation of **5**, as well as the increase in dispersity for those clusters encapsulated by peptides with greater degrees of rotational freedom. The complete analysis of the entire library is expected to produce important conceptual information about chelate features and lattice matching critical for the stabilization of CdS nanoclusters of specific size.

Notes and references

‡ The peptide (1 mmol) was disolved in 100 μL DMSO + 900 μL 0.1% TFA solution. In a 4 mL septum sealed vial, 250 μL peptide (1 mM) solution, 100 μL $CdCl_2$ (2 mM, 0.01 M HCl) solution and 1550 μL Tris (0.1 M, pH 8.5) were combined. With stirring, 100 μL aqueous Na_2S solution (2 mM) was added dropwise to the reaction mixture. After 4 h, the reaction solution was filtered through a 0.2 μm PVDF syringe filter for analysis.

§ The CdS lattice was constructed using the crystal builder feature of MOE (Chemical Computing Group, Inc., http://www.chemcomp.com). The lattice parameters used were space group $F\bar{4}3m$ with $a = b = c = 5.832$ Å and $\alpha = \beta = \gamma = 90°$. The unique Cd position was (0,0,0) and the unique S position was (0.25, 0.25, 0.25). The sphere was cut from a $6 \times 6 \times 6$ supercell using the proximity feature of MOE.

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