Relative Adsorption of Amine and Carboxylate Terminated PPI Dendrons to Gold Nanoparticles

Renée Suzich[1], Arunendra Saha-Ray[2] , Marie-Christine Daniel[2]

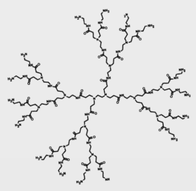
[1]Department of Biology, St. Mary's College of Maryland, 47645 College Drive, St. Mary's City, MD 20686

[2]Department of Chemistry and Biochemistry, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

Background

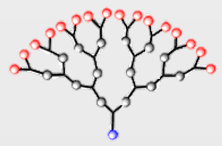
Nanovectors, including spherical metal nanoparticles and dendrimers, have the potential to greatly improve the toxicological profile and efficacy of chemotherapeutic drugs. By allowing drugs to be targeted to cancerous cells both actively and passively, nanovectors minimize unwanted side effects and maximize drug delivery. We coated gold nanoparticles (GNPs) with dendrons, combining the advantages of both systems.

Dendrimer



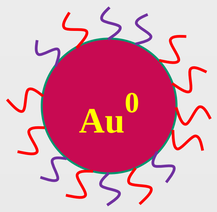
- Tunable surface chemistry
- Distinct exterior and interior chemical environments

Dendron



- Shape allows for coating of particle surface
- Disulfide linkage serves as attachment point to gold particle

Gold Nanoparticle

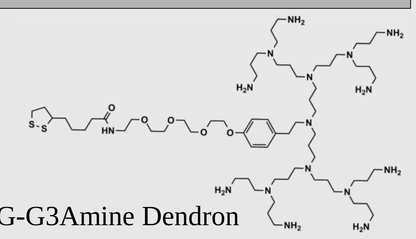


- Biocompatible
- Stable
- Size tunable

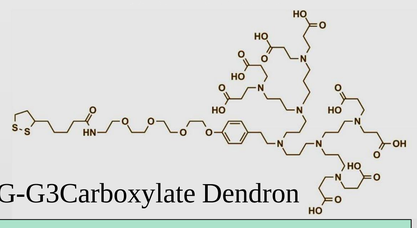
Project Overview

We coupled generation 3 amine and carboxylate-terminated PPI (polypropyleneimine) dendrons to 20 nm gold nanoparticle cores in order to determine the ratio in which the dendrons become bound to the core.

We used high-performance liquid chromatography (HPLC) and zeta potential **to assess the relative amounts of dendron attached** after etching the gold core with an iodine solution.



TA-TEG-G3Amine Dendron



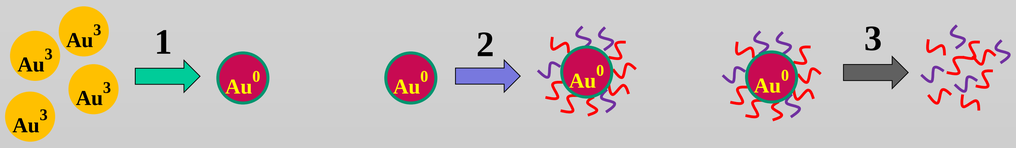
TA-TEG-G3Carboxylate Dendron

Methods

Gold Nanoparticle (GNP) Synthesis: 0.3 mM $HAuCl_4$ in water was refluxed with sodium citrate for 20 minutes in an oil bath at 115°C, using a 1:10 molar ratio of gold salt to citrate. **1**

GNP-Dendron Coupling: 15X excess dendrons was stirred with 20 mL GNP for 12 hours, centrifuged and dialyzed (12,000 MWCO) to remove excess citrate and dendrons. **2**

GNP-Dendron Etching: 200 µL of gold etch (1:4 potassium iodide/ iodine in water) was added to GNP-dendron solution and let stir overnight. The dendrons were recovered through dialysis (100-500 MWCO) to remove etched gold, followed by lyophilization. **3**



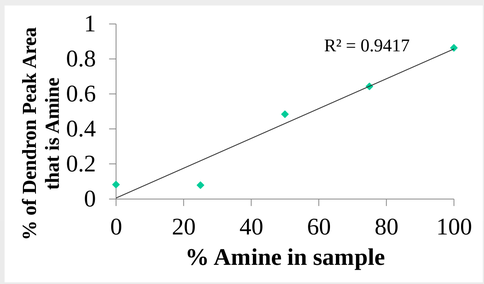
Analysis of Recovered Dendron with HPLC and Zetasizer: Reverse-phase HPLC was performed using a Jupiter C4 column (250 x 4.6mm, 300Å). The mobile phase was a 3 minute linear gradient beginning with 100:0 (v/v) water: methanol/isopropyl alcohol, and ending with 0:100 (v/v) water: methanol/ isopropyl alcohol followed by a 10 minute hold at these conditions. Flow rate was 1 mL/min and UV detection was performed at 280 nm. Zeta potential was measured with Malvern Zetasizer Nano ZS, all samples at pH 8.

* Calibration curves were constructed by creating mixtures of amine and carboxylate dendrons in ratios 0:100, 25:75, 40:60, 50:50, 60:40, 75:25, 100:0 respectively, and using peak areas from HPLC, and zeta potential values.
* Amine and carboxylate dendrons were added to GNP in ratios 0:100, 25:75, 50:50, 75:25, 100:0 respectively, and the dendrons recovered after etching were run through HPLC and Zetasizer to determine ratio of bound dendrons.

Results

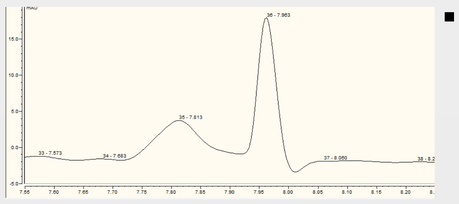
HPLC

Calibration Curve 20 mg/mL

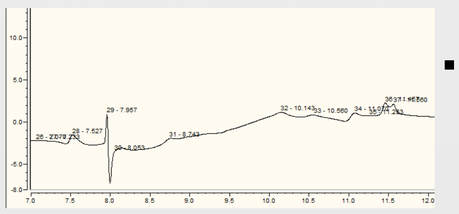


- Concentrations of 1 mg/mL and recovered dendron samples were not detectable
- Column became saturated at 15 mg/mL

50:50 Amine/COOH Dendrons Sample



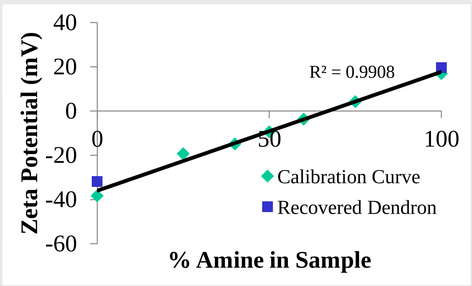
Recovered 100:0 Amine Dendron



- Broad amine dendron peak at 7.81 min and COOH dendron peak at 7.96 min of approximately equal areas
- Recovered dendron samples showed only contaminants

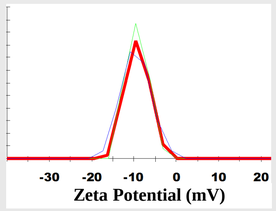
Zeta Potential

Calibration Curve at pH 8

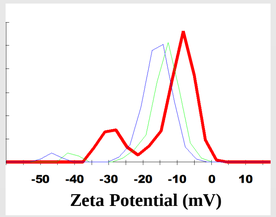


- The curve became parabolic if the carboxylate dendron was not deprotonated (at pH < 7)
- The recovered dendron samples from 100:0 and 0:100 amine/COOH coupling to GNP corroborate with calibration curve data

50:50 Amine/COOH Dendrons Sample



50:50 Recovered Dendron



- Preferable run overlap for calibration curve samples compared to recovered dendrons

Reaction Mixture (% Amine)	Coupled to Gold Core
0%	7.80%
25%	24.56%
50%	41.50%
75%	-0.01% *
100%	103.70%

- The dendrons coupled to the gold particles proportionally to their amounts in the reaction mixture, except when there was above 50% amine dendron.

Conclusion

- HPLC cannot be used to detect the recovered dendron from 20 mL GNP without pre-column derivatization of the dendron.

- Zeta potential can be used to determine dendron ratios of samples 0.2 mg/mL if the calibration curve is done at a pH of 7.5 or above, provided the recovered dendrons are run at the same pH.

- Carboxylate-terminated PPI dendrons may be preferentially adsorbed to gold particles when there is a mixture of 50% or more amine dendron with carboxylate dendron but further study is needed to rule out instrumental failing.

Future Work

* HPLC separation and column retention of dendron mixtures could be dramatically improved by splitting the samples through anion and cation exchange columns, although current selectivity from the C4 reverse-phase column is adequate for determining dendron ratios.
* Coupling the dendrons to GNP at different ratios (40:60, 10:90 etc.) could confirm the greater affinity of carboxylate dendron for GNP under certain conditions and elucidate the cause of this discrepancy.
* Improving the extinction coefficient of the dendrons, *e.g.* by coupling with FITC, may allow for HPLC quantification of recovered dendrons without necessitating a greater volume of GNP.

Acknowledgement

The project is supported by the National Science Foundation Research Experience for Undergraduates (REU) Research Award CHE-1460653 and made possible thanks to the mentorship and guidance of Dr. Marie-Christine Daniel, the synthetic work of Lance Dockery, and the help of all of the members of the Daniel lab.

* Result may have been influenced by instrumental error- Zeta cuvette electrode interaction with insoluble amine dendron