

# Elucidation of HEPES Affinity to and Structure on Gold Nanostars

Wenjing Xi<sup>®</sup> and Amanda J. Haes<sup>\*®</sup>

Department of Chemistry, University of Iowa, Iowa City, Iowa 52242, United States



### **HEPES**



- ✤ HEPES is a zwitterionic sulphonic acid buffering agent.
- ✤ It is named as 4 (2-hydroxyethyl) 1-piperazine ethanesulphonic acid.

It is widely used in cell culture, largely because it is better at maintaining physiological pH despite changes in surrounding media.

- ✤ It has high water solubility, stability towards low temperature.
- ✤ Ease of preparation.
- ✤ It act as metal-reducing, shape-directing and stabilizing agent.

# **Background work**

#### Geometry-Dependent Plasmonic Tunability and Photothermal Characteristics of Multibranched Gold Nanoantennas

Joseph A. Webb,<sup>†</sup> William R. Erwin,<sup>†</sup> Holly F. Zarick,<sup>†</sup> Jayde Aufrecht,<sup>†</sup> Harris W. Manning,<sup>†</sup> Matthew J. Lang,<sup>†</sup> Cary L. Pint,<sup>‡</sup> and Rizia Bardhan<sup>\*,†</sup>

<sup>†</sup>Department of Chemical and Biomolecular Engineering and <sup>‡</sup>Department of Mechanical Engineering, Vanderbilt University Nashville, Tennessee 37235, United States

#### Increasing Protrusion Length and Density (a) 1.2-(a) (c) -pH 7.1 10 minutes 0 minutes MGNs, mass = 184 µg - pH 7.44 Extinction (a.u.) 1.0 (i) -pH 7.69 55 -pH 8.03 -pH 8.2 (c) (c) 50 MGNs emperature 40 35 20 nm 10 nm 20 nm (iii) 20 nm 30 0.2-60 Water 30 mM 300 mM HEPES 1.0 ٠ 0.0 60 mM 500 600 700 800 900 1000 1100 120 400 100 mM 100 mM HEPES 50 5 10 Irradiation Time (minutes) Extinction (a.u.) - 160 mM (b) Wavelength (nm) (d) -200 mM (c) 60 ♦ 11.28 W/cm -300 mM 40 Sensitivity = 373 55 800 (mn) کک 4.84 W/cn 55 Peak Position (nm) (°) 50 760 Temperature (°C) 30 720-45 20 680 40 0.2 Sensitivity = 229 640 35 10 600 25 30 0.0 0 300 mM HEPES 20 560 25 1.35 1.40 1.45 100 mM HEPES 1.30 1.50 1000 40 80 120 160 200 400 600 800 1200 5 10 Irradiation Time (minutes) 520 Mass of MGNs (µg) η (RIU) 6.8 7.2 8.4 6.4 7.6 8.0 8.8 Wavelength(nm) pН

J. Phys. Chem. C 2014, 118, 3696-3707



pubs.acs.org/JPCC





## **Relevance to lab**



Anisotropic nanostructures synthesis in one step and seedless approach using such buffering agents.

Electrospray conditions can be used for synthesizing new materials using these pH dependent buffers.

# Label-Free SERS Detection



➢ Using SERS to understand how molecule interacts with metal postsynthesis.

Reconstructing ligand structures on NP surface.

# In this paper

• HEPES is used for formation and stability of Au nanostars (AuNS).

• Functional groups in HEPES (N, SO<sub>3</sub><sup>-</sup>, and OH) that bind to AuNS upon washing in acidic media are evaluated experimentally using localized surface plasmon (LSPR) spectroscopy, surface-enhanced Raman spectroscopy (SERS), zeta potential measurements and DFT calculations.

- Restructuring of AuNS morphology is illustrated upon acidification. It depends on pH of solution, acid composition and protonation state of HEPES.
- The surface sensitive technique, surface enhanced Raman scattering (SERS), reveals that pH variations induce reversible activation of the amine and sulphonate groups in HEPES, and that electron distribution weakens it affinity.
- Reversible surface activation of metal surface was used for SERS detection of benzene, a non-thiolated molecule that interacts with gold through London dispersion forces.
- This molecular-level insight illuminates how pH impacts the electronic structure of HEPES on AuNS. This is an important step forward for applications that involve these nanostructures synthesis.
- First example of directly detecting a nonthiolated molecule using these AuNS.

## **Results and discussion**



**Figure 1.** Plasmonic and structural properties of gold nanostars. (A) Representative LSPR spectra of 1.1 nM gold nanostars incubated in 1 mM HNO3 for (1) 0, (2) 0.5, (3) 1, (4), 2.5, (5) 3.5, (6) 5, and (7) 20 h. Spectra are arbitrarily offset for clarity. TEM images of nanostars incubated in pH (B) 7 and (C) 3 aqueous solutions for 24 h. (D) Evaluation of nanoparticle dimensions at pH 7 include the (B-1) ferret radius (22.9 ± 3.6 nm, N = 105), (B-2) branch length (14.0 ± 2.5 nm, N = 51), (B-3) radius of curvature of the tips (3.43 ± 0.44 nm, N = 148), and (B-4) core radius (8.1 ± 1.1 nm, N = 102), and at pH 3 after 24 h (C-1) average radius (13.2 ± 2.3 nm, N = 173) where N = number of measurements. (E) Evaluation of  $\Delta\lambda$ max for nanostars incubated in (a) 1 mM KNO3, (b) 1 mM KCl, (c) 1 mM HNO3, (d) 0.5 mM H2SO4, and (e) 1 mM HCl as a function of time.  $\Delta\lambda$ max represents a change from *t*0.



**Figure 2.** Mechanism of HEPES deprotonation (A) in aqueous solution and (B) on gold nanostructures. (C) Evaluation of 0.4 nM gold nanostar surface potential as a function of pH. The estimated surface pKa, 2 for N2 is 3.8 ( $\pm$ 0.2). Error bars represent the standard deviation of three replicate measurements. The error in pKa is estimated from uncertainty in linear analysis. Insets show DFT-optimized geometries and electrostatic potential maps of HEPES when the N2 amine is (1) deprotonated and (2) protonated.

### **Evaluation of HEPES interactions to Au nanostars**



**Figure 3.** Representative (A) LSPR and (B) SERS spectra for (1) 1.1 nM Au nanostars in 10 mM HEPES (1) before ( $\lambda$ max,1 = 679 nm) and (2) after washing with water ( $\lambda$ max,2 = 675 nm). (C) A normal Raman spectrum of 1 M HEPES is shown. The scale of the inset is magnified 30 × . Raman collection parameters:  $\lambda$ ex = 785 nm, *t*int = 25 s, *P* = 28 mW, average = 3.



**Figure 4.** Representative (A) SERS, (B) inverse second derivative, and (C) first derivative spectra of residual HEPES on 1.1 nM gold nanostars in aqueous solution. Panels A and B: pH = (1) 3.0, (2) 3.5, and (3) 7. Panel C: pH = (1) 4.5, (2) 3.8, (3) 3.5, (4) 3.2, (5) 3.0. Vibrational band assignments are found in Table 1. (D) pH-dependent vibrational frequency shifts relative to solution for the (1) SO3– asymmetric and (2) C–N stretches are shown. Raman collection parameters:  $\lambda ex = 785$  nm, *t*int = 25 s, *P* = 28 mW, average = 3.

#### **Implications of HEPES Disruption on the SERS Detection of Benzene**



**Figure 5.** SERS analysis (inset-inverse second derivative) of (A) 10 mM benzene incubated with 1.5 nM nanostars for 30 min after an initial 10 min pretreatment in water with a pH of (1) 3, (2) 3.5, (3) 4.0, (4) 4.5, (5) 5.5, and (6) 7.0. (B) SERS spectra (inverse second derivative) of 5 mM benzene after (1) pretreatment in pH 3 water for 10 min, (2) step 1 + neutralization for 5 min, (3) steps 1 + 2 + 1, and (4) steps 1 + 2 + 1 + 2. (C) Relative integrated areas of the ring breathing mode for benzene (1006–936 cm–1) to the asymmetric sulfonate stretch are shown. This ratio is reported relative to the first data point (pH 3 in cycle 1). Vibrational band assignments: 1360 (HEPES, SO3 asymmetric stretch), 964, and 979 cm–1 (benzene, ring breathing). Raman parameters:  $\lambda ex = 785$  nm, *t*int = 25 s, *P* = 28–58 mW, average = 3.



**Figure 6.** Benzene adsorption to 1.1 nM gold nanostars. (A) Representative SERS spectra of (1) 5, (2) 2.5, (3) 1.5, (4) 1.0, and (5) 0.2 mM benzene incubated with nanostars for 30 min after an initial 10 min pretreatment. Trends in signals for (B) benzene features centered at (1) 964 ( $\pm$ 0.5) cm–1 and (2) 979 ( $\pm$ 1.2) cm–1 and (C) HEPES at (1) 1351 ( $\pm$ 0.8) cm–1 and (2) 1378 ( $\pm$ 0.9) cm–1. Fits for panels B and C are from eq 2. Raman parameters follow:  $\lambda$ ex = 785 nm, *t*int = 25 s, *P* = 28 mW, average = 3.

# Conclusions

- ✓ Binding affinity and structure of HEPES on AuNS are largely influenced by solution pH.
- ✓ Variation in pH affects the morphology, surface potential, and surface activity of the nanostars as well as their subsequent use in SERS detection of benzene.
- ✓ Changes in electron distribution of HEPES were confirmed using DFT, SERS and LSPR.
- ✓ This work suggests that SERS analysis of small molecule stabilizing agents depends on many factors including electron redistribution in stabilizing agents and surface availability.
- ✓ Similar structural trends were observed for other buffering agents like MOPS and EPPS.

# Thank You