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Short communication

# Bisection of biotinylated soft spherical structures

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ABSTRACT

Biotin, a water soluble vitamin, upon conjugation with ditryptophan methyl ester affords soft spherical structures in the nanometer to micrometer range. The evolution of such morphology is ascribed to favorable interaction between biotin and tryptophan residues as suggested for high affinity biotin–avidin interaction. We report unprecedented observation that biotinylated soft spherical structures undergo bisection to reveal hemispherical capsule-like structures, in the presence of avidin**.** This study suggests singular approach of tuning bioinspired morphologies via natural principles of interaction and affinity. We envisage that such hemispherical capsules provide an expeditious entry into interesting soft structures which may be harnessed for potential applications.



## 1. Introduction

Avidin tenaciously binds to biotin with an affinity constant of ~ $10^{15}$ $M^{-1}$ and the half-life of avidin–biotin complex is determined to be ~200 days at the neutral pH [1–4]. An intricate participation of hydrogen bonding and hydrophobic interactions is attributed for the high affinity constant observed of the biotin–avidin complex. As a result of selectivity and high affinity, this interaction is frequently used for various applications in chemistry and biology [5].

We have recently reported formation, morphological studies and focused ion beam (FIB) milling of soft spherical structures obtained from the self-assembly of biotinylated Trp–Trp dipeptide methyl ester **1** (Fig. 1A,B) [6]. It was proposed that hydrophobic tryptophan residues would form the core of these soft structures, while exposing biotin moieties at the surface of the spherical structures. This possibility provided us an impetus to detect and confirm the presence of surface exposed biotin moieties through their interaction with avidin.

## 2. Materials and methods

### 2.1. Preparation of avidin-coated copper stubs

1, 2- Five copper stubs (2×5 mm) were cleaned with 12 no. silicon carbide armory paper, washed with water, methanol and acetone, followed by drying with air blower. The treated copper stubs were dried with compressed nitrogen and incubated with 1 mg/mL avidin solution at pH 7.1 in 10 mM phosphate buffer for 12 h at 4 °C. After that copper stubs were washed three times with deionized water and dried with compressed nitrogen. 20 μL of 1 mM solution of biotinylated di-tryptophan methyl ester **1**, biotinylated di-tryptophan acid, biotinylated di-phenylalanine methyl ester and biotin solution was put separately on the avidin-immobilized copper stubs and incubated at room temperature in a humid and dust-free environment for 1 h. The unbounded vesicles were then washed away with 50% HPLC grade methanol:water and the copper stubs were dried with nitrogen.

### 2.2. Fluorescence optical microscopy

10 μM fluorescein dye solution was added directly to avidin solution (1 mM). This solution was added to biotinylated conjugate **1** (1 mM) as a fresh sample or it was co-incubated for 24 h in 50% methanol/water. 20 μL of this solution was spread on a glass slide, dried at room temperature, and the dye stained structures were examined under imaged under fluorescence microscope (supporting information). The images were taken at maximum possible resolution of hemispherical structures (supporting information), and one of such image was digitally magnified to measure representative void diameter.

## 3. Results and discussion

First, we decided to probe the accessibility and relative affinity of biotinylated soft structures towards avidin by employing 2-[(4-hydroxyphenyle)azo]benzoic acid (HABA) assay [7–9]. This dye structurally differs from biotin, binds to (strept)avidin with a much lower affinity ($K_a = 10^4$ $M^{-1}$), thus gets quantitatively displaced from

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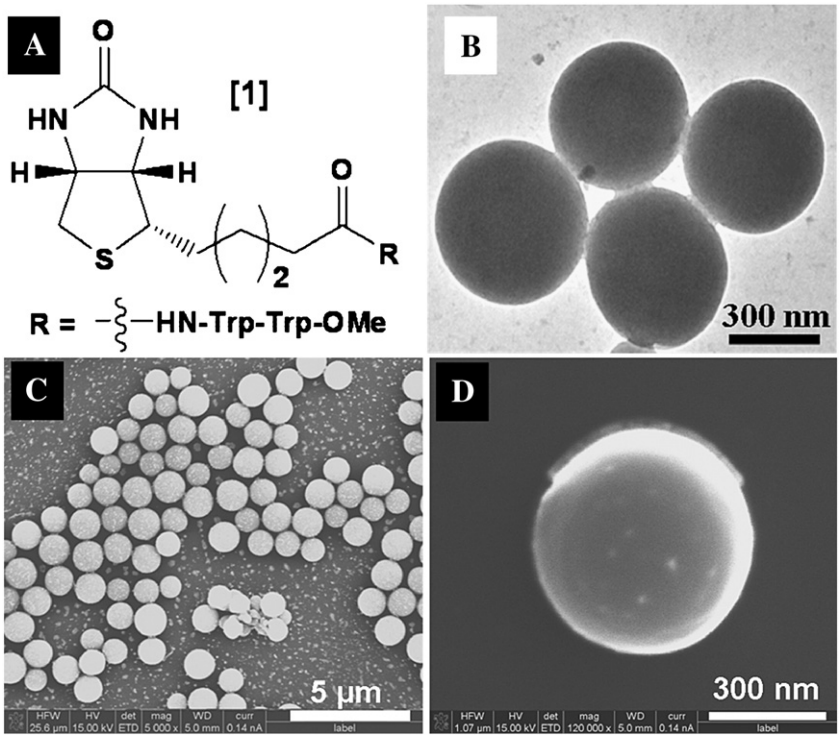


**Fig. 1.** Nature of self-assembled biotinylated di-tryptophan methyl ester (**1**). (A) molecular structure of **1**, (B) High resolution transmission electron micrograph (HRTEM) of spherical structures from **1**, (C) High Resolution Scanning Electron (HRSEM) micrograph of vesicles of **1** on graphite surface and (D) HRSEM micrograph of an intact vesicle.

its avidin complex, upon biotin binding. This observation suggests that both ligands perhaps share a common binding site. The binding of preformed vesicles of **1** and avidin was determined by HABA-assay where the absorption maximum of the latter (348 nm) is red-shifted to 500 nm upon binding to avidin [9,10]. Titration of a solution of HABA-saturated avidin with **1** resulted in a decrease in the UV/Vis absorbance at 500 nm, as expected for HABA displacement from biotin binding sites in avidin (Fig. 2). This observation confirmed that interacting biotin moieties are indeed located at the surface of the spherical structures and are able to freely interact and compete with HABA for avidin binding. Similar changes in the absorption were also noted when biotin alone was added to HABA-saturated avidin suggesting that spherical structures of **1** perhaps bind to avidin in a fashion similar to free biotin (Appendix A). On the other hand, biotin saturated avidin did not show any changes upon addition of **1** under UV/Vis spectroscopic observation (data not shown). The above result also indicates that the binding mechanism of **1** with avidin is similar to that of biotin binding mechanism.

Interestingly, the addition of avidin to a solution of preformed vesicles of **1** resulted in uniform bisection of soft spherical structures as determined by scanning electron microscopy (Fig. 3A). The evolution of new morphologies was slightly dependent on the concentration of added avidin (Appendix A). A similar change in morphology was also observed when a 1 mM solution of **1** was exposed to avidin coated copper stubs (Fig. 3B), thus indicating that this phenomenon occurs in solution as well as on surface.

The spectroscopic HABA-avidin complex assay demonstrated that binding mode of **1** with avidin was similar to biotin–avidin interaction. Thus, it is likely that that tenacious binding of avidin with spherical structures of **1** may facilitate bisection of these spherical structures. This observation also indicates the presence of highly dense biotinylation at the surface and that all biotin moieties are available to complex with avidin. Excess avidin either in solution or coated on the copper surface did not lead to further disruption of hemispherical structures. It was not possible to determine the stoichiometry and affinity of **1**-avidin complex, however our results suggest that the addition of biotin-saturated avidin to **1** does not alter shape of spherical structures as the biotin binding sites of avidin are occupied by native biotin.

We further decided to visualize these bifurcated structures in 3-dimension with a high resolution scanning electron microscope (HRSEM). An HRSEM micrograph of bisected vesicles, on a stage tilted at a 52° angle with respect to an incident electron beam, showed that the bisected vesicles possessed a bowl-like shape with a slight depression in the middle (Fig. 3C).

An image of fluorescein stained sample of **1**, when incubated with avidin, also corresponded well with the HRSEM data (Fig. 3D). The diameter of the void on the bisected vesicle was estimated to be ~230 nm from HRSEM (Fig. 3C, depicted by an arrow), was close to

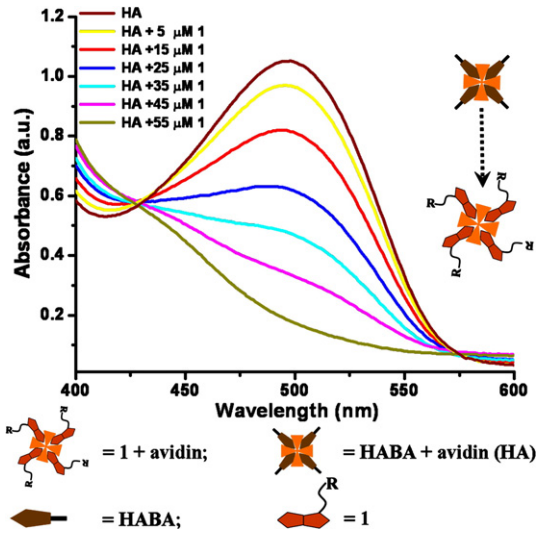


**Fig. 2.** Absorption spectra of back-titration of avidin-HABA complex with preformed vesicles of **1**.

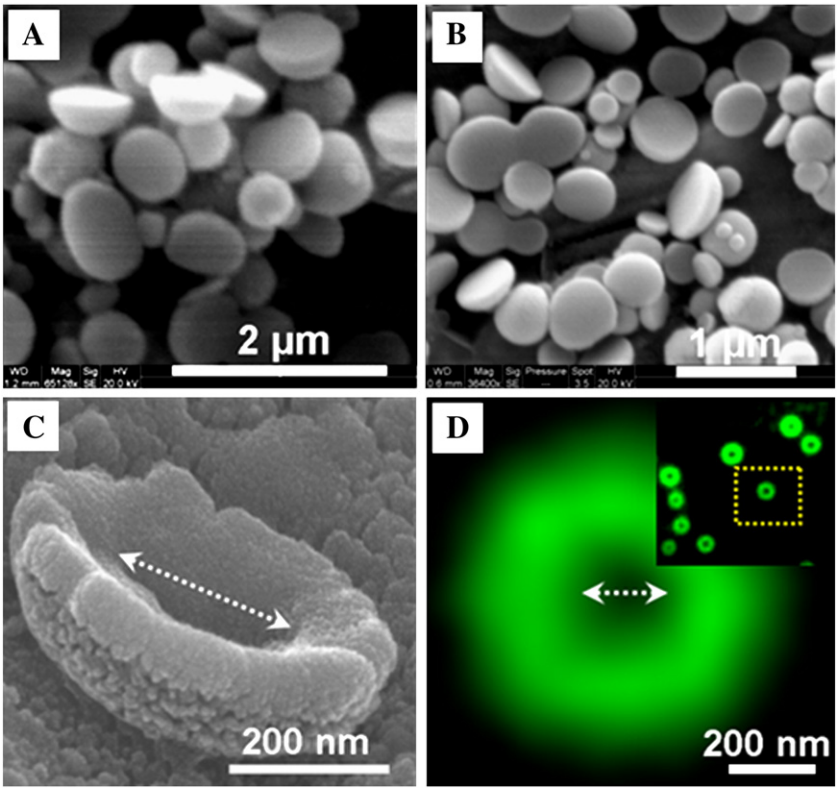


**Fig. 3.** Scanning electron micrograph of (A) bifurcated vesicles after adding the avidin into 1:1 ratio to solution of preformed vesicles of **1** (B) 1 mM solution of compound **1** on avidin coated surface (C) HRSEM micrograph of avidin added 1 mM solution of **1** into 1:1 ratios showing bowl shaped bifurcated vesicles and (D) fluorescent micrograph of co-incubated sample of **1** with avidin and fluorescein dye (inset: micrograph of fluorescein labelled multiple soft structures).

the void diameter deduced from the fluorescence microscope image of a bisected vesicle (Fig. 3D, ~200 nm, depicted by an arrow). This observation also confirms formation of bisected spherical structures that are corroborated by two independent microscopy analyses.

A schematic proposal in Fig. 4 depicts the proposed bisection pathway biotinylated soft vesicles with the help of avidin interaction. It appears that high affinity interaction between biotin moieties displayed at the spherical surface and avidin, in solution or when coated on surface, preferentially leads to pulling apart of these soft structures. Avidin–biotin interaction has been implicated for biomimetic membrane rupture or growth of adhesion plaque due to a spreading process [11]. Another study has shown transformation of vesicles to tubular structure via avidin–biotin interaction [12]. Finally, this interaction has also been implicated for the altered shape of biotinylated lipid vesicles and polypeptide-lipid microbubbles [13–17].

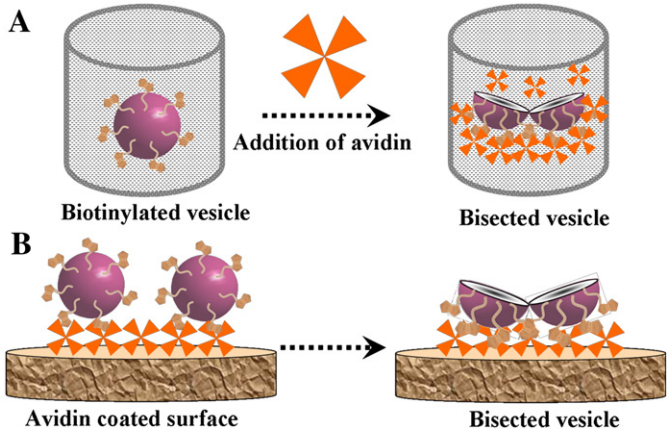


**Fig. 4.** Schematic representation of avidin assisted bisection of vesicular structures.

## 4. Conclusion

In summary, we have presented a unique and intriguing example of avidin-assisted bisection of biotinylated spherical structures. It is proposed that such interactions may serve as morphological triggers to alter the overall shape of soft structures. We plan to explore the generality of this observation to obtain a deeper insight and a detailed understanding of the bisection mechanism and to evaluate the utility of hemispherical structures obtained from the bisection process. It is envisaged that such hemispherical capsules provide an expeditious entry into interesting soft structures with bioessential components which may be harnessed for potential applications.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bpc.2008.11.016.

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