Chiroplasmonic DNA Scaffolded "Fusilli" Structures

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Supporting Information

1. Materials

Sodium cloride, Magnesium acetate, Bis(*p*-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt, and Tris(2-carboxyethyl)phosphine hydrochloride were purchased from Sigma–Aldrich. Gold colloid (AuNP) 10 nm was purchased from British Biocell (BBI Solutions). Amicon ultra 100 kDa and 30kDa filtering tubes were purchased from Millipore. Disposable filter units were purchased from Whateman GmbH. All DNA oligonucleotides were purchased from Integrated DNA Technologies. DNA sequences for the quasi-ring strands and thiolated strands were HPLC-purified, all other sequences were ordered as desalted material. Ultrapure water from a NANOpure Diamond (Barnstead) source was used in all experiments. Tris Acetate EDTA buffer (TAE) 50X and Tris Borate EDTA (TBE) buffer 5X were purchased from Biological Industries (Israel).

2. Instruments

High resolution transmission electron microscopy images were taken with a MagellanT XHR SEM (FEI). Circular dichroism spectra were obtained with a Jasco J-810 spectropolarimeter. Atomic force microscopy (AFM) characterization was performed with a Bruker Multimode V and ScanAsyst-Fluid+ probes. Cary 60 UV-Vis spectrophotometer (Agilent) was used to collect absorbance data. The self-assembly procedure was carried out using a Thermostatic bath Heto CBN 8-30.

3. Methods

3.1 Sequence Design

Initial design of the nanotube sequences was carried out by using CadNano2 software (http://cadnano.org/blog/cadnano-version-220), following a reported architecture.[1] Positioning of the promoting strands (**Ps**) and tethers (**T**) for the AuNPs, was achieved taking into consideration that (1) each quasi-ring is carrying one T for a total of 6 AuNP tethers per repeating unit; (2) the strands **T** must be at a distance of 16 bp moving from one quasi-ring position to the next; (3) the

positioning of the tethers **T** in the nanotubes are repeating for each nanobarrel unit; (4) positioning of the sequences **Ps** followed the idea that a staple sequence, at the crossover junction, must be pointing parallel to the surface of the nanotube and, following DNA duplex geometry, the 8th bp downhill the crossover junction must be pointing at an angle of 270° in respect to the surface of the nanotube; (5) **Ps** sequences at the 3' of the staple where the crossover is facing the upper quasi-ring are pointing out of the plane of the sheet while **Ps** sequences at the 3' of the staple where the crossover is facing the lower quasi-ring are pointing inside the plane of the sheet, while the opposite happens when the **Ps** elongating sequence is positioned at the 5' of the staple strand; (6) **Ps** and **T** sequences are positioned far away to avoid steric and/or electrostatic repulsion between **Ps** strands and the AuNPs and thus ensure high yields for the hybridization of the DNA modified AuNPs on the nanotubes; (7) special care was used to design the sequences joining the two quasi-rings R0-R5. This was due to the fact that the AuNP tethers **T** must follow, once the nanotube is formed, a full helix without defects. In order to accomplish this geometrical constraint, point (2) of this list must be applied carefully.

3.2 Nanotube Formation

The DNA nanotubes were formed by subjecting a 50 μl aqueous solution containing 100nM of each quasi-ring sequence and 500nM of each staple (see Table 2 for the list of specific staple sequences), 15 mM Mg(Ac)2, and 1XTAE buffer to a 95° for 5 minutes and then slowly decreasing the temperature to 20° in a thermostatic bath for 24 hours. The solution was then filtered using an Amicon 30 kDa molecular weight cutoff (MWCO) at 10000g for 5 min at 10° C.

3.3 AFM Characterization

The DNA nanotubes solution was deposited on a freshly cleaved mica support. After 10' minutes the surface was washed with water and dried. Imaging was performed in air, using the Bruker ScanAsyst software and the appropriate probe (ScanAsyst-Air), Figure S1.

3.4 AuNP Modification and Their Application to the Nanotubes

A published method was followed for the AuNPs modification.[2] In general, thiolated DNA strands were added to AuNPs to produce the DNA-AuNP hybrids. The yield of the single-modified nanoparticles was evaluated in previous publications and a ratio 1:1 of DNA to AuNPs was used.

1- AuNPs were incubated for 8 hours with an excess of bis(*p*-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt and then filtered using Amicon Ultra 100kDa MWCO device at 5000g for 3 minutes, at room temperature, to eliminate the excess of capping and concentrate the particles. The absorbance at λ=520nm and the AuNP extinction coefficient was used to estimate the concentration of the AuNPs. The particles protected with the phosphine capping were used as starting material for the DNA functionalization.

2- Thiolated DNA sequences **T_1'** and **T_2'** (15 μM) were incubated at room temperature for 1 hour with tris(2-carboxyethyl)phosphine hydrochloride (15mM) and then added to a AuNP solution containing 100mM NaCl, 0.5XTBE buffer, 0.5 mg/ml bis(*p*-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt. 1:1, DNA:AuNPs ratio was used. This solution was incubated for three hours at room temperature.

3- The particles were then mixed with a short thiolated DNA oligonucleotide (**Stab**) and the samples were incubated at room temperature for 48 hours, prior to filtering using Amicon Ultra 100kDA MWCO to eliminate the excess of HS-dT_5 (**Stab**).

4- To obtain the AuNP helices, 3μL of the assembled nanotube solution was mixed with the appropriate mix of **T'**-modified Au NPs, with a ratio 1:12, in a solution containing 15mM magnesium acetate in 0.5XTAE buffer for 48 hours at room temperature. The solution was then diluted in the same buffer to a volume of 120 μL (1nM concentration of the helices) immediately followed by CD and TEM characterization.

3.5 CD and TEM Characterization of the AuNP Helices

The samples were analyzed in a Jasco CD spectropolarimeter in a 1 cm light-path quartz cuvette and the CD spectra were corrected with a blank (20mM magnesium acetate in 0.5XTAE). Three signal accumulations for each experiment were averaged and the final spectra were smoothed. TEM characterization of the AuNP assemblies was performed with XHR-STEM Magellan (FEI). Few microliters of the AuNP-decorated nanotubes solution (1nM) were deposited on a TEM copper mesh and freeze-dried prior to imaging.

3.6 Computational modeling of the spectra

Lefthand helix Au nanospheres assembly was modeled in Matlab R2022b by means of Boundary Element Method simulations thanks to the libraries of Hohenester et al.[3,4] The 3D geometry was designed according to the TEM images of Figure 3, namely 100 Au particles of 16 nm in diameter assembled in helix with an overall diameter of 160 nm and 135 nm pitch. The simulated structure has a full extension of 215 nm along the z-axis. The dielectric environment was set as pure water. Extinction, absorption and scattering cross sections were therefore calculated as the average of 6 independent electromagnetic excitation, propagating along the x, y and z axis and polarized on the other two perpendicular axis, according to previously published procedures.[5–7]

Table S1. Quasi-ring DNA sequences. All sequences are listed in the 5'→3' orientation.

R0	TCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGGCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGT
R1	TGATTTATAAGGGATTTTGCCGATTTCGGAACCACCATCAAACAGGATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCGCTTGCTGCTATTCTTT
R2	CAGGGCCAGGCGGTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTAACTCTCT
R3	CGCAACGCAATTAATGTGAGTTAGCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAG
R4	CGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTC
R5	CCTCAACTGTGAGGAGGCTCACGGACGCGAAGAACAGGCACGCGTGCTGGCAGAAATATGACCATGATTACGAATTCGAGCTCGGTACCCGGGGAT

Table S2. Staple DNA sequences for building the nanotubes. All sequences are listed in the 5'→3' orientation.

Name	Sequence
1	AGTCCACTTGATGGTG-Ps
2	GCGGGGAGGCTGTTTC
3	AATCCCTTAGTGTTGT-Ps
4	CACAACATAGTTGAGGATCCCCGGGGAACAAG
5	CTGTGTGACGTAATCA-Ps
6	AGCCTGGGTGCCTGTT-Ps
7	GTCTATCATTTCTGCC
8	CTCCTCACACGAGCCGGAAGCATACCGCTTTC
9	GTTCCGAATTTCTTTT
10	CAAAGGGCGCTGGTTT-Ps
11	CTTCGCGTTCCAACGT
12	T_2-CAGTCGGGAGACGGGC
13	CATTAATTTGGCCCTGAGAGAGTTGCAGCAAG-T_2
14	ATGAATCGGGGCGCCA-Ps
15	TAGGGTTGATAAATCAAAAGAATAAGGCGGTT
16	AGCACGCGGTGCCTAA
17	CACCAGTGAAACCTGT-Ps
18	TGCGTATTGCCAACGC-Ps
19	TCCAGTTTGTACCGAG
20	Ps-TCCGCTCACTGCATTA
21	Ps-TGAGTGAGCTAACTCA
22	CGTGCCAGCAATTCCA-T_1
23	CGGTCCACGAAAAACC-Ps
24	TCACCGCCGCGTTGCGCTCACTGCAAGTGTAA
25	GCCCCAGCATTGCCCT-T_1
26	ATCCTGTTATTAAAGAACGTGGACCCGTGAGC
27	Ps-AACAGCTGAGGCGAAA
28	GGGTGGTTATCGGCAA
29	T_2-CTCGAATTAATTGTTA
30	TGGTCATAGCCCGAGA-T_1

Table S3. Tethers (T), promoter strands (Ps), and thiolated strands sequences (T_1' and T_2') for the surface modification of the AuNPs, and they are complementary to T_1, T_2, respectively; PsC sequence corresponds to the nucleic acid strand complementary to the Ps strands. Stab is the short thiolated strand for the stabilization to high salt concentrations of the AuNPs. All sequences are listed in the 5'→3' orientation.

name	sequence
T_1	ATATCTGTGAGCGGC
T_2	TCATGCATCTAGACT
T_1'	thiol-CCG CCG CCG CTC ACA GAT AT
T_2'	AGT CTA GAT GCA TGA GAT CT-thiol
Stab	thiol-TTTTT
Ps	TTTTTATTTTTATTTTTATTTTTA
PsC	TAAAAATAAAAATAAAAATAAAAA

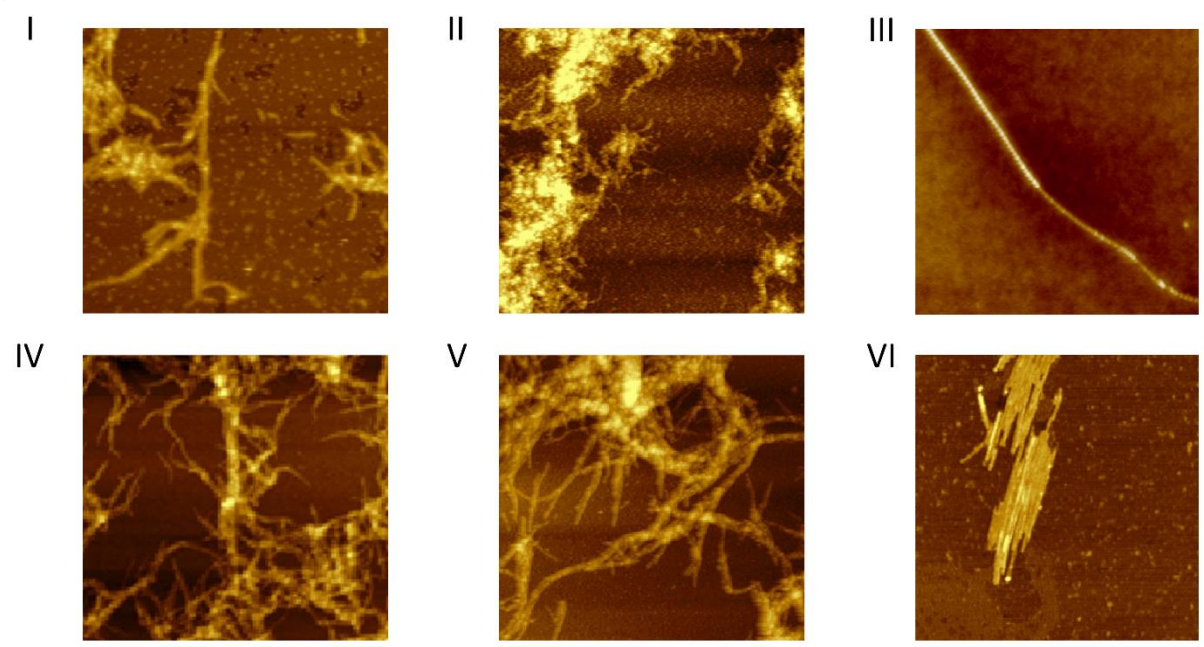

Figure S1. Magnified portions of AFM profiles shown in Figure 2A of the manuscript. Panels I to VI, corresponds to the same Panels of Figure 2A.

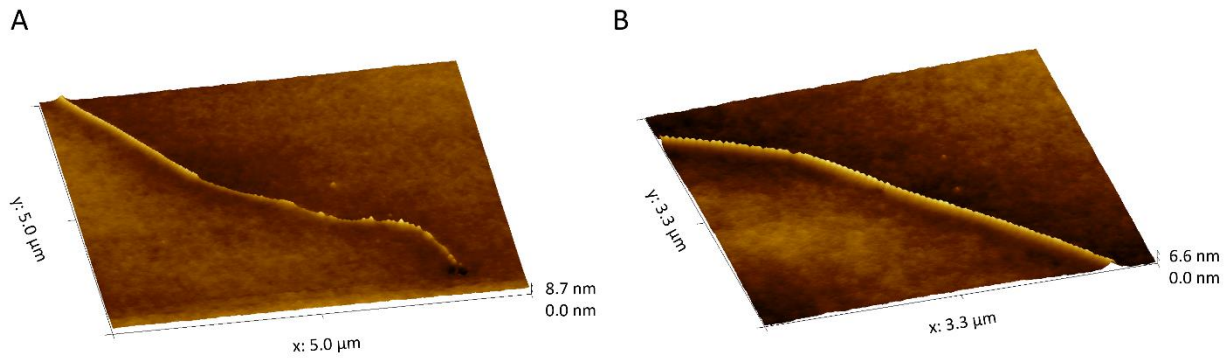

Figure S2. 3D representations of the DNA scaffold shown in Figure 1B (A) and Figure 2A, Panel III (B) of the manuscript.

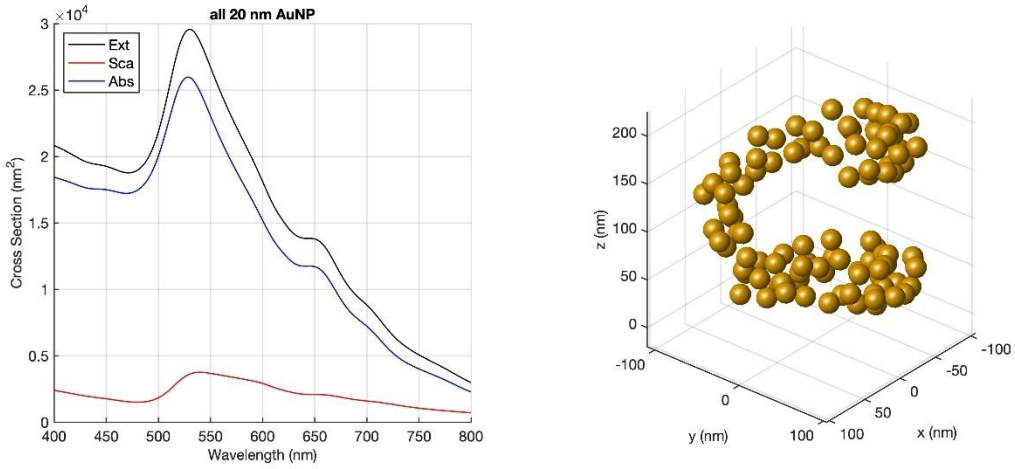

Figure S3. Simulated spectra and 3D scheme of the chiroplasmonic fusilli corresponding to the experimental one shown in Figure 5A.

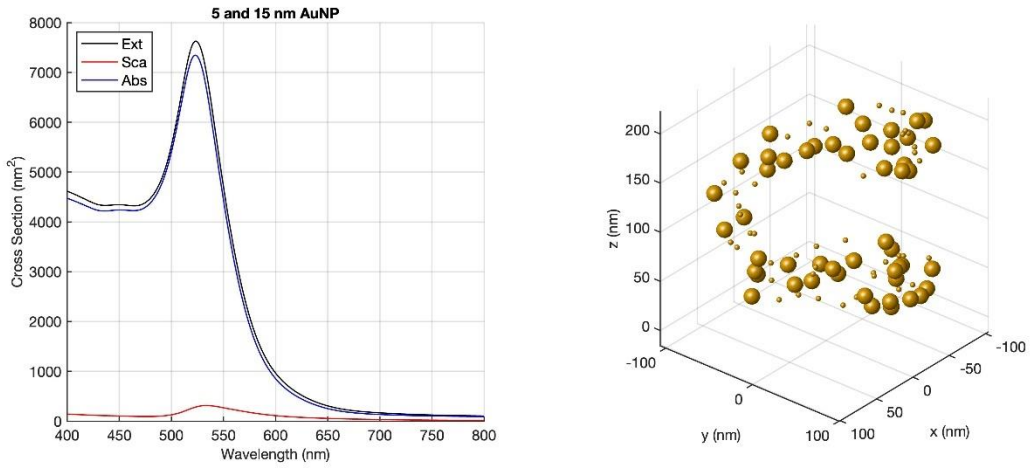

Figure S4. Simulated spectra and 3D scheme of the chiroplasmonic fusilli corresponding to the experimental one shown in Figure 5C.

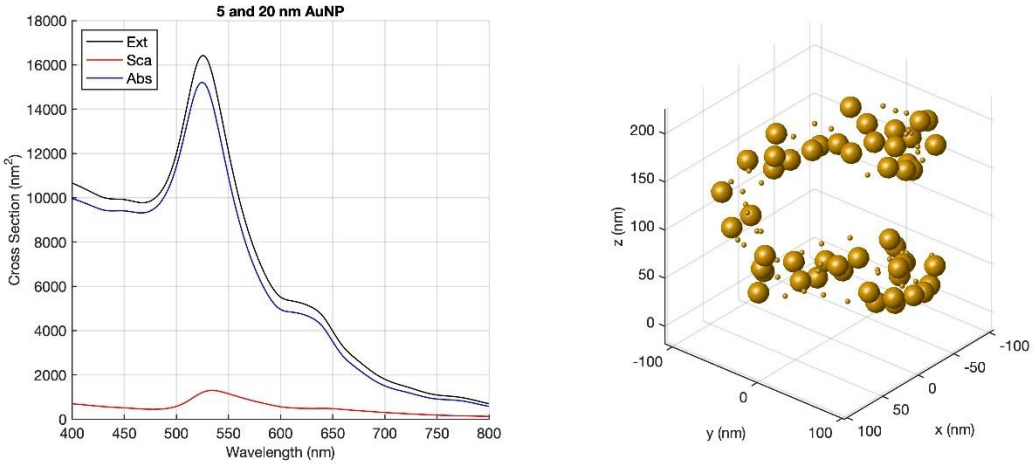

Figure S5. Simulated spectra and 3D scheme of the chiroplasmonic fusilli corresponding to the experimental one shown in Figure 5D.

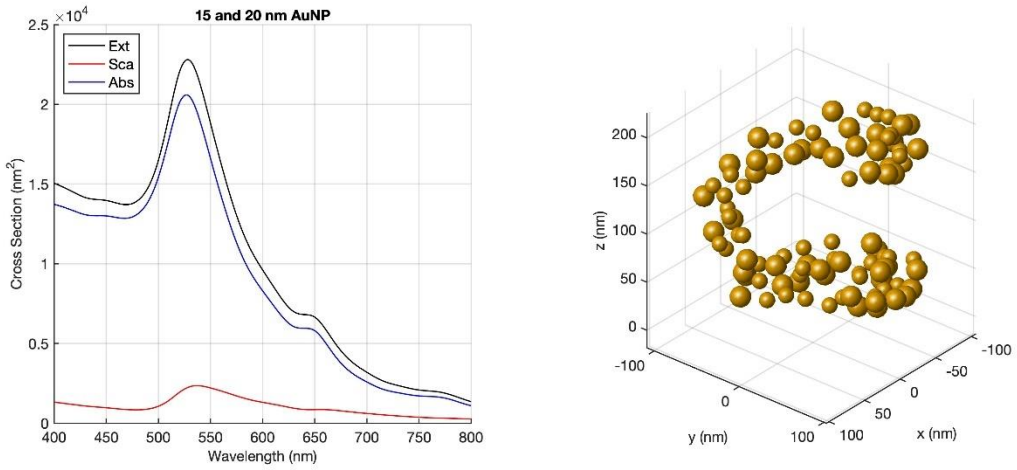

Figure S6. Simulated spectra and 3D scheme of the chiroplasmonic fusilli corresponding to the experimental one shown in Figure 5E.

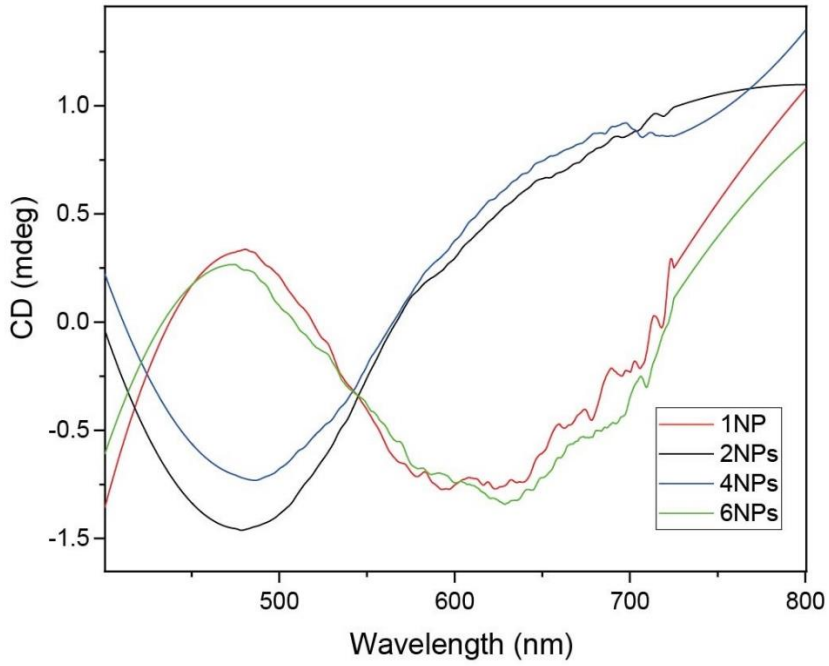

Figure S7. CD analysis of the DNA tubular scaffold modified with 1 AuNP per barrel unit (red line), 2 AuNPs per barrel unit (black line), 4 AuNPs per barrel unit (blue line), and 6 AuNPs per barrel unit (green line).

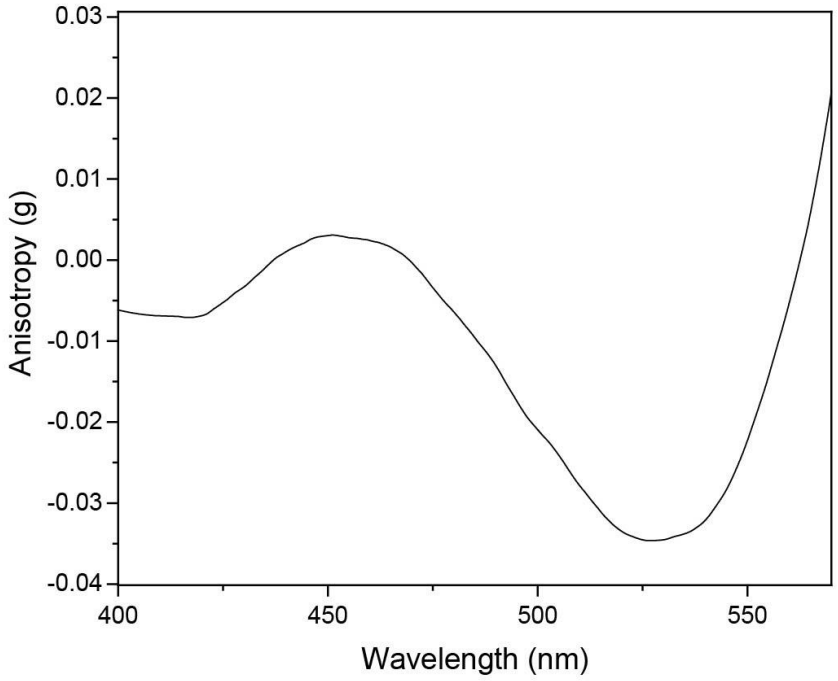

Figure S8. Anisotropy g-factor profile corresponding to the fusilli chiroplasmonic structure.

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