Supporting Information for

Determination of Surface Chirality by Near-Field Scanning Optical Microscopy

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Figure S1: Schematic illustration of the NSOM setup in the reflection configuration used in this work

Preparation of chiral surfaces of L or D Histidine crystals

Gold substrates: Microscope glass slides were immersed in a 1:1 mixture of ethanol:acetone and cleaned in an ultrasonic bath (70 °C) for one hour. Afterwards, the slides were placed in extra pure water and heated (70 °C) for an additional hour. The slides were removed from the water and blown dry with nitrogen. The gold substrates were prepared by the vacuum evaporation of 99.99% pure gold, carried out at a pressure of 5x10⁻⁶ Torr. First, a thin layer of chromium was sputtered (10 nm) and then a layer of gold (100 nm) with an evaporation rate of 0.01 nm/s.

Chiral L or D Histidine crystals

Analytical grade L and D Histidine (>99.9%) were purchased from Aldrich – Sigma. Their purity was checked by a polarimeter and they were used without further purification. All the crystallization experiments were conducted with supersaturated
solutions of the amino acid in deionized water at room temperature. Crystallization experiments were carried out in glass jars containing 10 mL at 25°C. 1 g of D or L Histidine was suspended in 10 mL of deionized water. The solution was heated to 70 °C and stirred for 15 min until it is completely dissolved, and then the gold surfaces were placed vertically in the crystallization solution. The solutions were left to cool spontaneously to room temperature. After ca. 10-12 hour's micrometer size crystals of L or D Histidine crystallized on the gold surfaces and near the edge of the surfaces. The gold surfaces were gently removed from the solutions dry under N₂(g) and used for NSOM measurements.

**Preparation of chiral self assembled multilayers of D or L Cysteine**

Gold substrates were immersed in 10 mM aqueous solution of L or D Cysteine for of 2 hours. Under these experimental conditions we achieved well-defined multilayers with a thickness of approximately 6 nm as confirmed by ellipsometry, XPS and X-ray diffraction measurements.