Combining high-resolution scanning tunnelling microscopy and first-principles simulations to identify halogen bonding

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Scanning tunnelling microscopy (STM) is commonly used to identify on-surface molecular self-assembled structures. However, its limited ability to reveal only the overall shape of molecules and their relative positions is not always enough to fully solve a supramolecular structure. Here, we analyse the assembly of a brominated polycyclic aromatic molecule on Au(111) and demonstrate that standard STM measurements cannot conclusively establish the nature of the intermolecular interactions. By performing high-resolution STM with a CO-functionalised tip, we clearly identify the location of rings and halogen atoms, determining that halogen bonding governs the assemblies. This is supported by density functional theory calculations that predict a stronger interaction energy for halogen rather than hydrogen bonding and by an electron density topology analysis that identifies characteristic features of halogen bonding. A similar approach should be able to solve many complex 2D supramolecular structures, and we predict its increasing use in molecular nanoscience at surfaces.
In order to engineer robust functional molecular nanostructures at surfaces, self-assembly via strong, directional intermolecular forces is often required. Both halogen and hydrogen bonding\(^2\) possess these characteristics to different extents, offering different potential nanofabrication pathways and allowing for a diverse set of molecular moieties that can participate in the formation of supramolecular architectures. There are many examples in the literature of two-dimensional (2D) hydrogen bonded (HB) assemblies on surfaces that have been examined with surface science techniques\(^3\)–\(^6\). Halogen-bonded (XB) molecular nanostructures\(^1\)–\(^7\),\(^9\) are considerably less common in on-surface 2D systems but are increasingly being studied and seen as an important addition to the ‘toolbox’ of supramolecular assembly. Typically, iodine\(^10\)–\(^14\) and bromine atoms\(^15\)–\(^19\) are used as halogen bond donors due to their strong polarisability, but examples of chlorine\(^20\) and fluorine\(^11,12\) based assemblies have also been reported. Halogen bonds can also involve halogen atoms acting simultaneously as both donor and acceptor\(^11,16,17,23,24\), with the positive electrostatic potential of the sigma hole oriented towards the central ‘belt’ of negative electrostatic potential found on an adjacent halogen atom. Alternatively, nitrogen\(^10,12,13,19\) and oxygen\(^17,25–27\) atoms act as acceptor sites in heteromolecular assemblies.

2D assemblies are commonly characterised on surfaces with scanning probe microscopy (SPM) techniques such as scanning tunnelling microscopy (STM) and atomic force microscopy (AFM). In recent years, higher resolution forms of SPM have become available\(^28–32\) that make use of functionalised tips (such as CO, Xe, D\(_2\), H\(_2\), Br, and CuO)\(^28,30,31,33,34\) to reveal the internal structure of molecules adsorbed on surfaces with astounding clarity\(^29\). Non-contact AFM (NC-AFM) and high-resolution STM (HR-STM) have been used to resolve the structures of molecules that are difficult to determine with more traditional analytical methods such as NMR or mass spectrometry\(^35–37\), as well as to identify intermediates and products of reactions that have taken place on surfaces\(^38–41\). In particular, NC-AFM and HR-STM have been employed for examining the internal structure of graphene nanostructures, as the number and type of molecular rings can easily be resolved\(^42–45\). There are also various examples of the use of these techniques for studying the supramolecular structure of 2D self-assembled layers\(^21,46–49\), although in most of these cases the chemical structure of the molecular components led to an unambiguous assignment of the type of intermolecular bonding.

Here we identify a case—the self-assembly of 3,9-dibromo-peri-xanthoxanthene (3,9-Br\(_2\)PXX) molecules on a Au(111) surface—where the potential coexistence of different intermolecular interactions presents a formidable challenge for standard forms of SPM. In particular, while conventional STM measurements cannot determine whether 3,9-Br\(_2\)PXX assemblies via HB or XB, only by means of HR-STM are we able to identify XB interactions as the driving force leading to the formation of supramolecular assemblies of 3,9-Br\(_2\)PXX on Au(111).

**Results**

**Molecular assembly.** 3,9-Br\(_2\)PXX (Fig. 1a) is a dibrominated derivative of PXX, an electron donor molecule\(^30–34\), derivatives of which have previously been employed as \(p\)-type semiconductors in organic thin-film transistors due to their efficient carrier injection properties, high mobility, and thermal stability\(^54,55\). The Br and O atoms in 3,9-Br\(_2\)PXX offer the possibility of both HB and XB intermolecular interactions when arranged into supramolecular arrays on a surface. HB can originate from non-classical weak C–H···O interactions, while the possibility of XB stems from the emergence of the so-called σ-hole\(^26\) on the Br atom (Fig. 1b). Density functional theory (DFT) calculations (see Methods) show that there is a region of negative electrostatic potential that forms a belt around the C–Br bonds, while a region of positive potential (the σ-hole) develops in the elongation of the same bond, thus providing the opportunity for Br and O atoms to act as halogen bond donors and acceptors, respectively.

At low coverages, the majority of the 3,9-Br\(_2\)PXX molecules self-assemble into kagome-type structures (phase 1) that develop in the face-centred cubic (fcc) regions and elbow sites of the Au(111) herringbone reconstruction (Fig. 2a). At 77 K, the peripheries of the kagome islands are often seen to vary between scans, indicating a significant level of molecular mobility. Combined with the lack of any noticeable herringbone distortion, we attribute this to the 3,9-Br\(_2\)PXX molecules being weakly bound to the Au(111) surface (typically, strongly bound adsorbates result in a lifting or a significant distortion of the herringbone reconstruction\(^57–60\)). Two mirror symmetric orientations of the kagome structures can be found, suggesting that the pro-chiral 3,9-Br\(_2\)PXX molecules segregate into chiral domains upon adsorption. The kagome structure itself can be thought of as being composed of triangular sub-units that consist of three molecules, each appearing to be bound with the end of one molecule pointing to the side of another (Fig. 2d). While this indicates that the oxygen atoms of 3,9-Br\(_2\)PXX are involved in intermolecular bonding, the nature of the interaction is not clear, as is discussed below. The triangular sub-units are packed into a hexagonal unit cell with lattice vectors \(a = b = 2.2 ± 0.1\) nm, and an angle of 60 ± 2°. Full unit cells are rarely found due to the confinement of the assembly in the fcc regions of the herringbone reconstruction.

Coexisting with the kagome structure, a second minority assembly (phase 2, Fig. 2b) is observed at low molecular coverage. Phase 2 consists of irregular structures that, similar to the kagome assembly, are limited to the fcc regions of the Au(111) herringbone reconstruction. Within these irregular islands, variations in molecular shape are observed, with some of the ends of the molecules appearing differently, and some molecules having an extra feature at the sides (Fig. 2e). Increasing the coverage results in the development of a further, denser assembly (phase 3, Fig. 2c), where the molecules are arranged into parallel rows (Fig. 2f). This structure forms extended and compact islands that span both fcc and bcc regions of the substrate and coexist with few remaining phase 1 and phase 2 regions (Supplementary Fig. 1).

Initial modelling of the kagome assembly by overlaying molecular structures onto the STM images results in two distinct possibilities for the type of intermolecular interaction that governs the assembly. The unit cell dimensions and possible
orientations of the molecule are compatible with either halogen bonding between the Br end groups and the O atoms of adjacent molecules (Fig. 3b), or a non-classical O···H–C hydrogen bond and, possibly, a secondary electrostatic Br···H interaction (Fig. 3c). The relatively featureless appearance of the molecules in typical STM tunnelling conditions does not present an obvious solution to this, even when varying the bias voltage. In this case, the position of the molecular groups (and thus the type of assembly) can therefore not be directly inferred by using standard STM techniques.

High-resolution STM imaging. In order to clarify the structure of the kagome assembly, we harnessed the capabilities of HR-STM, which we performed at 7 K using a CO-functionalised tip. In agreement with previous HR-STM experiments and theoretical studies, intramolecular features could be resolved when approaching the CO tip close to adsorbed molecules (Fig. 4). The rings of the molecule (in particular, the naphthalene aromatic sections) are clearly resolved, as are the positions of the C–Br end groups. This allows us to overlay a molecular model of 3,9-Br₂PXX in a single orientation for all molecules in the kagome structures of a given chirality (the flipped orientation of 3,9-Br₂PXX matches the HR-STM images of molecules in the kagome structures of the opposite chirality). By doing so, it appears evident that in the triangular sub-units of the kagome packing the Br atoms of one molecule directly face the O atoms of a neighbouring one, as would be expected in XB (Figs. 4d and 3b). Moreover, overlaying scaled molecular models gives an estimated O···Br distance of 3.1 ± 0.1 Å and a C–O···Br angle of 176 ± 2°. The former is smaller than the sum of the van der Waals radii of the two elements (3.37 Å) while the latter is very close to 180°, thus satisfying two established criteria for identifying halogen bonds. All examples of the kagome packing studied by HR-STM were determined to be governed by halogen-bonding interactions. No examples of hydrogen-bonded assemblies could be found, demonstrating that the 3,9-Br₂PXX molecules on Au(111) form only XB at low coverages.

DFT calculations and image simulations. To get further insight into the nature of the intermolecular bonding, XB and
only displays the wrong symmetry with respect to the experimentally observed assembly (Supplementary Fig. 6) but also suggests that the position of the atoms in the HB assembly would produce markedly different features in the HR-STM images. In particular, the bonding region in the XB assembly is characterised by two brighter triangular features crossed by dark streaks (Fig. 5b), while the HB assembly would have an overall darker appearance in (Fig. 5c). We note that the darker streaks observed between the bromine and oxygen atoms of adjacent molecules (Figs. 4c and 5b) should not be interpreted as the ‘imaging’ of the halogen bonds, as it has been demonstrated that such intermolecular features can result from the relaxation of the flexible CO probe.

Analysis of the electron density topology. The DFT calculations allows us also to investigate the electron density topology which, in conjunction with the structural features discussed earlier, provides an additional probe to determine the presence of an intermolecular XB. To this aim, we have examined the electron density $\rho$ in the proximity of the O and Br atoms, which is plotted in Fig. 6a as a contour map projected onto the molecular plane. It can be clearly recognised that a gradient path connects O and Br and that a saddle point, corresponding to the bond critical point (BCP), is located in between O and Br. This is another determining feature listed in the IUPAC definition of halogen bond. Moreover, Fig. 6b shows the computed bonding charge density difference, i.e. the electron density difference $\Delta\rho = \rho_d - \rho_{m1} - \rho_{m2}$, where $\rho_d$ and $\rho_{m1,2}$ refer to the electron density of the dimer and the monomers, respectively. One can notice the emergence of some charge transfer from the Br atom to the O atom, with a net accumulation of electron density (red regions) along the direction of the O–Br bond path. Also this in agreement with the fact that “the forces involved in the formation of the halogen bond are primarily electrostatic, but polarisation, charge transfer, and dispersion contributions all play an important role”. We finally note that the charge density redistribution is not limited to the close proximity of the O and Br atoms but is characterised by a rather complex pattern as could have been expected from the highly non-uniform molecular electrostatic potential in Fig. 1b. This results in the formation of a sort of binding pocket rather than a single point contact.

Identification of synthetic impurities. HR-STM also helps to elucidate the nature of the minority phase 2 by revealing the internal structure of the molecules with an “unusual” appearance and demonstrating that these differ in their configuration of C–Br bonds from the expected 3,9-Br$_2$PXX structure. In particular, Supplementary Fig. 2 shows that some of these molecules have one terminal C–Br group shifted by one position (i.e., they are 2,9- and 2,8-dibromo isomers), while others are instead tri-bromo-minated, with an extra Br atom covalently bound to a carbon on the side of the molecule (1,3,9-Br$_3$PXX). The intermolecular interactions controlling the assembly of phase 2 are mostly XB, with some possible contribution from non-classical O–H–C hydrogen bonds and secondary electrostatic Br–H interactions (Supplementary Fig. 2). Although all the analytical spectroscopic characterisations suggested that the 3,9-Br$_2$PXX obtained was pure (Supplementary Figs. 11–14), small traces of dibromo isomers and tribromo derivatives might still have been present in the sample due to their low solubility (see experimental procedures in the Supplementary Methods). The fact that these impurities appear in our STM study at a relatively high concentration may be due to a higher rate of sublimation than 3,9-Br$_2$PXX, resulting in an overrepresentation when deposited onto the surface. In any case, the ability of clearly identifying these molecules by
HR-STM when other, conventional spectroscopic characterisation techniques fail to do so, is a further demonstration of the potential of sub-molecular resolution scanning probe microscopy as an analytical tool for chemical structure determination.

Interestingly, the minority phase 2 appears to be made exclusively of defective molecules (of various types), while the regular 3,9-Br2PXX molecules all assemble into the kagome phase 1 structures.

Finally, HR-STM also shows that the compact phase 3 that develops at a higher molecular coverage is kept together by synergistic HB and XB (Supplementary Fig. 3). The molecules are arranged into parallel rows and interact with each other via non-classical O–H–C hydrogen bonds as well as type I halogen bonds between terminal C–Br groups. Interestingly, this assembly is identical to that characterising the 3D crystalline phase of 3,9-Br2PXX as determined by X-ray diffraction (Supplementary Fig. 4), with one of the lattice parameters being slightly larger, most probably due to the molecule-surface interaction. Phase 3 is almost exclusively composed of regular 3,9-Br2PXX molecules, although occasional defective molecules (both 2,9-Br2PXX and 2,8-Br2PXX dibromo isomers and 1,3,9-Br3PXX tribromo derivatives) are observed within the compact islands, and partially de-brominated molecules are sometimes found at the edges (Supplementary Fig. 5).

In conclusion, high-resolution STM performed with a CO-functionalised tip is found to be necessary to conclusively identify the arrangement of dibrominated sp2-hybridised molecules within an on-surface self-assembled molecular network. The experimental information provided by HR-STM provides the basis for a theoretical structural, electrostatic, and electron density topology analysis, allowing us to identify with great accuracy the emergence of halogen bonding as the intermolecular interaction that stabilises the observed molecular structures. Whereas standard metallic-tip STM is unable to differentiate between halogen and hydrogen assembly motifs, HR-STM clearly identifies Br–O halogen bonding as the only source of the observed kagome assemblies of 3,9-Br2PXX on Au(111). DFT calculations support this result by predicting a higher stability of halogen versus hydrogen intermolecular binding while simulations of the HR-STM images demonstrate a remarkable agreement with the experimental data only for the halogen bonding interaction. HR-STM also identified the presence of defective molecules that escaped the scrutiny of traditional analytical methods and thereby fully solved the experimentally observed 2D self-assembly. Building upon the cases in which HR-SPM has been shown to be an invaluable tool for identifying the chemical structure of unknown molecules or for determining the intermediates and products of on-surface reactions, this work exemplifies the unique analytical insight that HR-SPM can give in establishing the nanoscale arrangement of molecules within a supramolecular structure and the nature of the intermolecular interactions ruling the self-assembly. We believe that this methodology can be effectively used when investigating 2D networks originating from the self-assembly of planar molecules on surfaces and that a significant fraction of difficult or controversial molecular structures that have been discussed in the literature over the last decades could be quickly and clearly solved by using this approach.

Methods
Scanning tunnelling microscopy measurements. STM experiments were performed on a low temperature STM under ultra-high vacuum (UHV) conditions. The Au(111) single crystal was cleaned with multiple cycles of Ar+ sputtering and annealing. 3,9-Br2PXX was deposited via sublimation (483 K) onto the Au(111) crystal, held at room temperature. The crystal was then cooled to 77 K or 7 K for
STM analysis. Standard STM measurements were carried out with bias voltages (V, applied to the sample) in the range of ±2.0 V and tunnelling currents of 50–200 pA. In order to perform high-resolution STM experiments, CO was leaked into the UHV system, adsorbed onto the Au(111) crystal held at ~10 K, and picked up by the STM tip. High-resolution STM images were taken in constant height mode (current channel). STM image analysis was performed with WSxM65, Gwyddion66 and LMAPper67.

Density functional theory calculations. DFT calculations were performed using the mixed Gaussian and Plane-Waves (GPW) method implemented in the CP2K package68. As the description of both halogen bonding (XB) and hydrogen bonding (HB) interactions is known to be quite sensitive to the choice of the exchange-correlation (XC) functional69,70, we have used two differently self-consistent non-local XC functionals, namely vdW-DF370 and optB86-vdW71, to assess the reliability of our results. Goedecker-type pseudopotentials33 with four, one, six and seven valence electrons for C, H, O and Br respectively have been employed. The Kohn-Sham orbitals were expanded in a Double-Zeta Valence plus Polarisation (DZVP) Gaussian-type basis set, while the plane wave cutoff for the total energy of 3 meV/atom.

Image simulations. The simulated HR-STM images have been obtained thanks to the PP-AFM/STM framework of Hapala et al.64 and Krejčí et al.81. The CO-functionalized tip employed experimentally is approximated by a probe particle bonded to the STM tip: this bond is 4 Å long and characterised by a lateral stiffness of 0.5 N/m. The charge of the probe particle is set equal to zero. As the experimental HR-STM images were obtained in constant height using very low bias voltages, all HR-STM simulated images were calculated as constant height dI/dV maps at the energy of the 3.9-Br-PX molecules, thus totalling 96 atoms, together with a vacuum region of ~20 Å inserted between the periodic replica of the 2D self-assemblies (along the direction normal to the assemblies planes) is sufficient to ensure an accuracy of the resulting images.

Further details on the STM methods as well as the full synthetic details are given in the Supplementary Methods.

Data availability
All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Information. Crystallographic data (excluding structure factors) for compound 3.9−Br−PX reported in this paper have been deposited at the Cambridge Crystallographic Data Centre, under deposition number 1901257. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request cif. Additional data supporting the findings of this study are available from the corresponding author upon reasonable request.

Code availability
The electronic structure calculations presented in this work have been obtained by the CP2K (version 4.1) package, which is an open source code freely available at https://www.cp2k.org. The simulated HR-STM images have been computed via the PPSTM framework of Ondřej Krejčí, which is freely available via GitHub at https://github.com/ondrejkrejci/PPSTM.

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Author contributions
J.L. performed the surface deposition experiments, the STM and HR-STM experiments, analysed the experimental data, and wrote the first draft of the manuscript. H.P. performed DFT calculations. G.C.S. performed the electronic structure calculations, computed the simulated HR-STM images and analysed the results. L.D. and D.B. designed, synthesised, and characterised the molecular module. G.C. conceived and coordinated the research project. All authors participated in discussing the data and editing the manuscript.

Competing interests
The authors declare no competing interests.

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