

# Understanding the Effects of Sample Preparation on the Chemical Structures of Petroleum Imaged with Non-contact Atomic Force Microscopy

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**ABSTRACT:** This study addresses the effect of sample preparation conditions on the structural integrity and composition of heavy hydrocarbon mixtures imaged by non-contact atomic force microscopy (nc-AFM). We designed and prepared a set of organic molecules mimicking well-accepted key characteristics of heavy oil asphaltenes including molecular architecture, molecular weight, boiling point, atomic H/C ratio and bond strength. We deliberately focused on multi-core molecule structures with long aliphatic linkers as this architecture was largely absent in previous nc-AFM studies of petroleum samples. The results confirm that all these molecules can be

successfully imaged and remain intact under the same preparation conditions. Moreover, comparison with ultra-high resolution FT ICR-MS of a steam-cracked tar asphaltene sample suggests that the single molecules identified by nc-AFM span the entire molecule spectrum of the bulk sample. Overall, these results suggest that petroleum molecules within the scope of chosen molecules studied herein can be prepared intact and without bias and the imaged data can be representative.

## **Introduction**

A better understanding of the molecular structures in the complex heavy fractions of crude oils is a formidable scientific challenge and is of great importance to the petroleum industry.<sup>1</sup> Despite decades of effort in academia and industry using cutting-edge ultra-high resolution FT ICR MS,<sup>2</sup> advanced chromatography,<sup>1</sup> and compositional modeling,<sup>3</sup> the specific structure of the majority of heavy petroleum molecules eluded direct characterization until now. Importantly, non-contact atomic force microscopy (NC-AFM) demonstrated atomic-resolution on individual organic molecules.<sup>4</sup> Therefore, applying NC-AFM on petroleum molecules is appealing. Many petroleum molecules have since been imaged and their chemical structures have been fully or partially resolved with NC-AFM,<sup>5-6</sup> demonstrating the power and versatility of this technique in addressing various problems of interest to the petroleum industry; however, questions remain to fully understand the more subtle details in these chemical structures. Of particular importance in this study is the impact of the sample preparation procedure on the AFM imaging results; especially on the thermal crackability and volatility of the presumed multi-core petroleum asphaltene molecules under the flash heating conditions used.

The sample preparation in NC-AFM involved the use of resistive flash heating (increase to a few hundred or a thousand degrees Celsius within a second) under ultrahigh vacuum ( $\sim 10^{-10}$  mbar).<sup>5, 7-8</sup> *A priori* it is unclear if this procedure is biased against certain kinds of molecules in petroleum since molecules with high enough molecular weight are expected to fragment.<sup>9</sup> Heavy molecules such as asphaltenes are typically featured by high boiling points and molecular weights, low H/C atomic ratio ( $\sim 1.1$ ) and polar molecules enriched in heteroatoms.<sup>1</sup> Another general concern is that this methodology may favor the transfer and imaging of lighter molecules over heavier molecules that remain behind due to their high boiling points and low volatility. To address all these concerns, we designed a few model compounds with two aromatic cores (pyrene) connected by hydrocarbon linkers with molecular properties resembling those of heavy oil molecules. In a previous study, we characterized the aliphatic moieties as part of organic molecules with AFM imaging.<sup>10</sup> In this study, we clarified the accessible preparation window and confirmed that within this window no cracking or bias was caused by the sample preparation.

### **Experimental and computational details:**

NC-AFM measurements: details of the instrument and measurements have been described elsewhere.<sup>4-6, 10-11</sup> Briefly, the data was collected with a home-built combined STM (scanning tunneling microscope) and AFM setup operated under low temperature (4.7 K) and ultrahigh vacuum ( $\sim 10^{-10}$  mbar) conditions. A qPlus sensor functionalized with a carbon monoxide tip was operated in the frequency-modulation mode,<sup>4, 12</sup> and AFM measurements were acquired in constant-height mode at zero bias voltage. The solid material was introduced by mechanically depositing a small amount ( $\sim 1$  mg) of the sample on a piece of Si wafer by a H-torch annealed glass pipette. The surface of the wafer featured a thin native oxide layer and the wafer was flash-heated by resistive heating in front of the cold substrate (at  $\sim 10$  K) which is composed of a Cu(111)

single crystal partially covered with bilayer NaCl.<sup>4</sup> The cold surface has virtually a 100% adsorption efficiency and prevents agglomeration of molecules by diffusion.

Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT ICR MS): measurements were conducted on a 15 Tesla Bruker solariX XR FT ICR MS (Bruker Daltonics Inc., Billerica, MA, USA). A solution in toluene at a concentration of 25-100 ppm was prepared and injected into an atmospheric pressure photoionization (APPI) source. Nitrogen was used as both the nebulizing and the drying gas. Data acquisition was set between  $m/z$  300 and 3000 range with an accumulation time between 40 and 60 milliseconds. Data analysis was performed with Bruker Data Analysis (DA) software and calibrated with an internal homologous mass series.

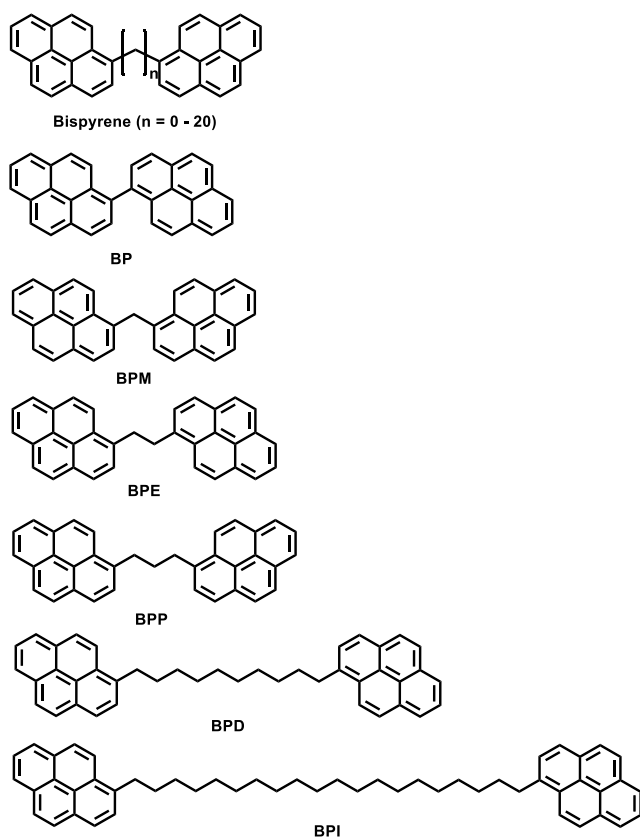
Computational method: Ground-state geometry optimizations were completed with Becke's three-parameter hybrid exchange functional with the Lee–Yang–Parr correlation functional (B3LYP)<sup>13-14</sup> and Perdew and Wang's 1991 gradient-corrected correlation functional (PW91)<sup>15</sup> as implemented in Gaussian 09 Revision D.01.<sup>16</sup> Vibrational frequency analyses were performed to verify that the stationary points correspond to energy minima (zero imaginary vibrational frequencies) or transition states (one imaginary vibrational frequency). All calculations were done in the gas phases.

## Experiment Design

A series of compounds composed of two pyrene units linked by a hydrocarbon chain of various lengths was designed and synthesized (**Chart 1**) to understand the effects of sample preparation in NC-AFM on the imaging results. These were inspired by early studies on related or similar compounds,<sup>17-19</sup> after surveying numerous model compounds employed in the literature. These compounds were designed to mimic heavy hydrocarbons in petroleum, particularly asphaltenes, based on molecular weights, boiling points, H/C ratio, number of carbons, double bond equivalent

(DBE) and most importantly, the types of chemical bonds which encompass most abundant carbon-carbon bonds in the hydrocarbon space. We note that there is no consensus on specific structures and properties of asphaltenes as these are defined by their (in)solubility in specific solvents rather than by their chemical structures.<sup>20</sup> We focused on hydrocarbon molecules in this study because they are the backbones of petroleum molecules. Furthermore, hydrogen and carbon account for the majority of petroleum elemental composition (>85% in most crude oils), although it should be noted that heteroatoms often play a significant role in many physical properties.

Chart 1. A series of organic compounds designed with pyrene to represent typical polynuclear aromatic hydrocarbons, and a hydrocarbon linker of various carbon chain lengths (0 – 20).



### **(a) The effect of molecular weight**

The molecular weight distribution of petroleum, especially for the high boiling point fractions, has caused significant confusion due to the molecular diversity and aggregation of these molecules. Thanks to advances in ionization techniques and the intrinsic ultrahigh resolution achieved by FTICR-MS, the molecular weight profile has now been established to lie between several hundreds to a few thousands, peaking around 600-700 in most studies on heavy fractions (asphaltenes and vacuum residua).<sup>21-22</sup> The compounds in this study were designed to have a molecular weight range from 400 – 700, covering the majority of heavy hydrocarbon molecules in petroleum. Previously, many organic molecules were imaged with NC-AFM, including aromatic hydrocarbons of high molecular weight, such as 522 (hexabenzocoronene) and 720 ( $C_{60}$ , buckminsterfullerene),<sup>11</sup> 973 ( $C_{78}H_{36}$ , cloverleaf),<sup>9</sup> 1802 ( $C_{140}H_{120}$ , wheelbarrow),<sup>23</sup> and 200 – 700 for coal asphaltenes;<sup>5</sup> however, the effect of molecular weight is still not certain, and it has been generally believed to be limited to ~1000 because decomposition or fragmentation of larger molecules has been observed.<sup>9, 23</sup> Therefore, this series of compounds can be considered as a gauge of the effect of sample preparation on measured molecular weight.

### **(b) The effect of boiling point**

In the context of AFM imaging results, a frequently raised concern is how quantitative and representative is the flash heating procedure in vaporizing heavy petroleum molecules onto the substrate kept at cryo temperature (5 K) without alteration. Heavy molecules in petroleum, such as the residue from vacuum distillation around 538 °C (1000 °F) in a refinery, or solvent-extracted asphaltenes, are often associated with high boiling points. The model compounds were designed to have boiling points in this range in order to mimic the behavior of petroleum molecules under the same flash heating conditions. Measurements of boiling points with high temperature simulated

distillation confirmed that the boiling points of these bispyrene compounds range from 1000 °F (538 °C) to 1180 °F (638 °C), with an estimated error of up to  $\pm 2$  °C (**Table 1**). It should be noted that the determination of boiling points higher than this range is problematic due to the likely thermal cracking reactions which start around 350 – 400 °C under normal conditions (under atmospheric pressure or vacuum distillation) for most organic compounds. Synthesis of compounds of high molecular weight becomes a significant challenge, due to the typical low solubility for large aromatic hydrocarbons. Indeed, the low solubility of BPI in chloroform presented significant issues in chromatographic purification and NMR spectroscopic characterization. It is expected that heteroatoms commonly contained in heavy fractions could significantly increase the boiling points due to non-bonded secondary interactions between polar groups. Therefore, these hydrocarbon molecules should help confirm the results for heavy molecules with boiling point higher than 538 °C (1000 °F) in petroleum fractions.

Table 1. Molecular properties of model compounds in Chart 1 indicate that the molecular weights and boiling points are representative of vacuum residue species.

Name	Compounds	Formula	Mol. Wt.	H/C	Purity % wt.	b.p. °C (°F)
BP	1,1'-bipyrene	C <sub>32</sub> H <sub>18</sub>	402.50	0.56	99.5	538 (1000)
BPM	di(pyren-1-yl)methane	C <sub>33</sub> H <sub>20</sub>	416.52	0.61	96.1	553 (1027)
BPE	1,2-bis-(1-pyrenyl)ethane	C <sub>34</sub> H <sub>22</sub>	430.55	0.65	97.3	555 (1031)
BPP	1,3-bis-(1-pyrenyl)propane	C <sub>35</sub> H <sub>24</sub>	444.58	0.69	98.3	571 (1059)
BPD	1,10-bis-(1-pyrenyl)decane	C <sub>42</sub> H <sub>38</sub>	542.77	0.90	95.0	592 (1098)
BPI	1,20-bis-(1-pyrenyl)icosane	C <sub>52</sub> H <sub>58</sub>	683.04	1.12	96.3	638 (1180)

Boiling point can be used as a proxy descriptor for the flash sublimation which transfers the single molecules to the surface to be analyzed. Even though the samples do not melt or boil during preparation, boiling point provides a common basis for comparing the response of various heavy molecules to sample preparation. Boiling point also allows the sample preparation for AFM measurements to be correlated to typical refinery distillation. The judicious choice of these probe molecules also test the hypothesis if the apparent concentration of multicore molecules is reduced by their lower vapor pressure due to entanglement of linker chains.

### (c) The effect of bond strength

Although many types of chemical bonds are possible in petroleum molecules, three types of carbon-carbon bonds are of primary interest:  $sp^2-sp^2$ ,  $sp^3-sp^3$ , and  $sp^2-sp^3$ , which differ significantly in bond energy. By varying the spacer length between the pyrenes, we also tested the variation in CC bond energy as a function of distance from the pyrene core, with an intention to cover all three types of bonds. Five bond strengths can be distinguished due to electronic effects: (a) The CC bonds between the  $\beta$  carbon atoms and further in BPI and BPD have typical CC bond strength (89 kcal/mol). (b) The CC bonds between  $\alpha$  and  $\beta$  carbon atoms in BPP, BPD, and BPI (each molecule has two) are weaker ( $\sim 75$  kcal/mol) due to the presence of benzylic radical formed. (c) The central CC bond ( $\alpha-\alpha$ ) in BPE is the weakest bond ( $\sim 65$  kcal/mol), due the production of two benzylic radicals. (d) The CC bonds in BPM  $sp^2-sp^3$  are quite strong (100 kcal/mol) due to the  $sp^2-sp^3$  bond. (e) The central aryl-aryl bond of BP ( $sp^2-sp^2$ ) is the strongest bond ( $\sim 120$  kcal/mol).

Table 2. Bond dissociation energies (BDE, kcal/mol) for typical carbon-carbon bonds in petroleum hydrocarbons show good agreement between experimental measurements and calculations.



C-C bond	Experimental <sup>a</sup>	B3LYP/6-31+G(d)	B3PW91/6-31+G(d)
CH <sub>3</sub> -CH <sub>3</sub>	90.1 (0.1)	85.8	87.4
PhCH <sub>2</sub> -CH <sub>3</sub>	77.1 (0.6)	70.4	72.5
Ph-CH <sub>2</sub> CH <sub>3</sub>	102.2 (0.7)	93.3	94.6
Ph-Ph	118 (1)	108.7	109.9
Ph-CH <sub>2</sub> Ph	97 (1)	80.3	82.4
PhCH <sub>2</sub> -CH <sub>2</sub> Ph	65.2 (0.9)	55.2	58.0

<sup>a</sup>Experimental bond enthalpies in kcal/mol and their uncertainties.<sup>24</sup>

These bond strength predictions were based on experimental data from literature<sup>24</sup> and calculations with density functional theory (**Table 2**) on a simpler benzene version instead of pyrene for computational efficiency. It was found that calculations at B3LYP/6-31+G(d) underestimate bond dissociation energy (BDEs) by 4-17 kcal/mol, which is slightly improved by the B3PW91 method, although the BDE trend is correctly predicted by both level of theories. Collision-induced dissociation (CID) experiments showed that BPE can be fragmented at a much lower energy (eV) and to a greater extent than that of BPD and BPI, confirming the weak bond strength in BPE.

Therefore, the series of compounds in this study covers all of the CC bonds present in petroleum, with a wide range of 65 – 120 kcal/mol. The weak bond in BPE is particularly interesting, because it is most likely to crack under the same thermal conditions. Few CC bonds are weaker than this, such as the central CC bond in 1,1',2,2'-tetraphenylethane, but these structures are uncommon in petroleum molecules. This series of model compounds should be able to verify the imaging results for multicore structures under the same conditions, and the variable bond strengths can function as a *molecular thermometer* imbedded in high molecular weight hydrocarbons to gauge flash heating conditions. If petroleum molecules undergo thermal cracking reactions during flash

heating, the CC bonds would fragment into radical species. If the weak bonds break, these free radical species could be observed by AFM imaging because of reduced diffusion coefficients at low temperatures (5 K). For example, many short-lived reactive intermediates including free radicals<sup>5, 25</sup> and aryne<sup>26</sup> have been successfully imaged by AFM.<sup>27</sup> It should be noted that the analysis above only considers the types of chemical bonds, which is only a kinetic factor during thermal cracking, but does not take into account the number of each type of chemical bond, which would be required for a full statistical analysis. For example, the naphthenic rings (five or six-membered aliphatic ring) can be considered as two CC bond linkers and should be covered within the scope of molecules studied. Herein, we only focus on the qualitative cracking mechanism in this study and defer the quantitative analysis to future studies (as well as other possible reactions such as aromatization).

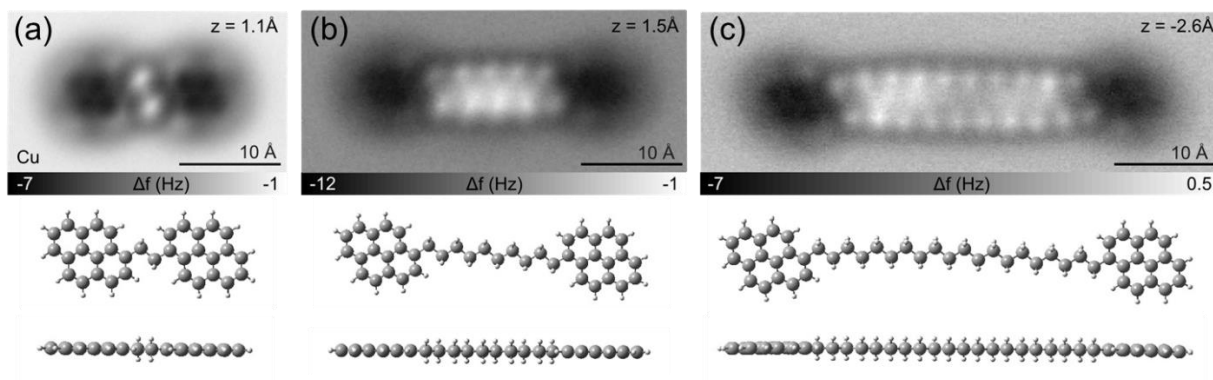
As discussed above, the considered range of molecular weights (400 – 680), boiling points (550 – 650 °C), and bond strengths (65 – 120 kcal/mol) of the model compounds is wide enough to include the majority of heavy oils and even a significant fraction of asphaltenes. For example, these compounds have H/C ratios from 0.56 – 1.12, consistent with expected H/C ratios for asphaltenes which are characterized by highly aromatic molecules with H/C ~1.1 or less. Additionally, these molecules have double bond equivalents (DBEs) of 24 and carbon numbers from 25 – 50, consistent with the reported values of asphaltenes in the literature.

## **Results and Discussions**

### **(a) Designed compounds can be imaged intact without fragmentation or cracking with AFM imaging**

Due to the complexity and challenge for sample preparation and the NC-AFM measurement, and the necessary time to image a large number of molecules, plus the expected nonplanarity for

BP, BPM, and BPP (Supporting information, Figure S1-S5), only three compounds were selected (BPD, BPI, and BPE) for imaging (**Figure 1**).<sup>10</sup>

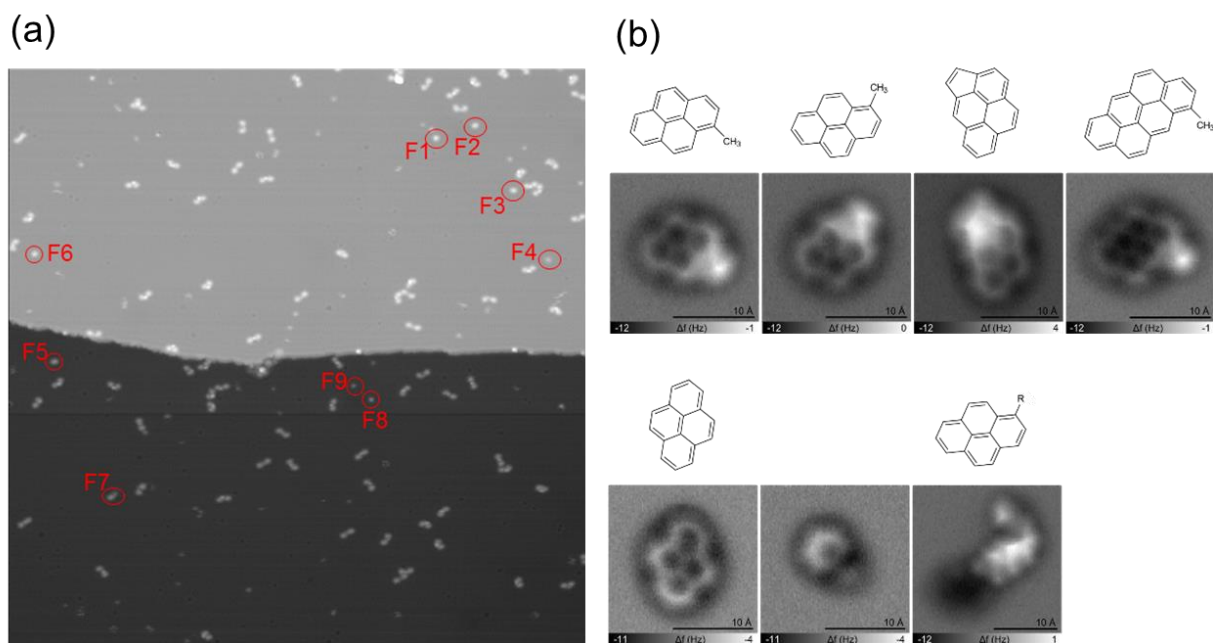


**Figure 1.** The AFM images of BPE (a), BPD (b) and BPI (c) and their corresponding molecular models. Adapted from Ref. 10 published by The Royal Society of Chemistry.

As shown in a previous report and **Figure 1**, all three compounds were prepared separately and imaged by NC-AFM. No fragmentation was observed in any of the compounds, despite BPI having a high molecular weight (680), boiling point (638 °C) and expected low volatility, and BPE containing a weak  $sp^3$ – $sp^3$  bond. This observation is consistent with previous studies.<sup>7-8, 19</sup> Tip manipulation further confirmed that the CC bond in BPE is indeed weak, because it could be purposely broken by a voltage pulse (about 3V) between tip and sample, while the same manipulation does not break the CC bonds in BPD and BPI. This showed that weak bonds up to 65 kcal/mol could withstand the same flash heating condition. Many conformations are observed in these compounds because of the flexibility of its carbon chains; however, no aggregation (agglomeration or self-association)<sup>28</sup>, or  $\pi$ - $\pi$  stacking of the two pyrene units were observed at this low coverage. From this study, petroleum molecules with similar molecular weight 300-700 and boiling point of up to 650 °C (1200 °F) range are reasonably expected to be imaged intact in the same fashion, regardless of the presence of chemical bonds as weak as 65 kcal/mol.

## (b) Representativeness of imaging results from purity analysis of model compounds

BPD could be stably imaged with scanning probe microscopy. However, some molecules assigned as impurities were observed in addition to the BPD molecules (**Figure 2**). We believe these molecules are more likely impurities in the synthetic source material rather than new products formed during evaporation because these observed structures are not free radicals as would be expected from thermal cracking in UHV and unlikely rearrangement products (same molecular formula after reaction). Furthermore, within a random area (120 nm x 120 nm) selected in the STM view, 81 BPD molecules were easily identified, and 9 impurity molecules were found and their structures characterized. The purity of BPD on the surface by counting molecules is ~90%, while the weight purity was found to be 95% by including the molecular weight of impurities, in good agreement with the purity from bulk analysis (LC-MS). From this study, the data suggests that the imaged molecules are representative of the overall sample composition. In addition, it shows that this technique can characterize impurities present in the analyte, potentially shedding light on the mechanism of their formation during synthesis.



**Figure 2.** (a) STM image of a random area (120 nm x 120 nm) after the BPD sample prepared on the surface, with impurities labeled in red circles (the rest of molecules are BPD). (b) Structures of impurities characterized with NC-AFM. Adapted from Ref. 10 published by The Royal Society of Chemistry.

**(c) Comparison of imaging data on a heavy oil mixture with bulk analysis.**

Steam-cracked tar was produced by steam cracking of crude oils, and the asphaltenes were isolated from this tar by heptane precipitation (10/1 v/v) with a yield of 24.1 wt%.<sup>6</sup> The assigned structures from the AFM imaging (**Chart 2**) provide an opportunity to cross-check the fidelity of our sampling by calculating bulk properties from the assigned structures. For example, the elemental composition and atomic H/C ratios from bulk analysis (Supporting information, Table S1) of the same tar sample are fairly consistent with those calculated from the AFM imaged structures, despite some presumptive assignments having been made to resolve ambiguities in the images for bulky side groups and heteroatoms.<sup>6</sup>

Chart 2. Structures of steam cracked tar molecules revealed by NC-AFM imaging, with some simplifications (shown in red) made from original assigned structures, in order to calculate their molecular weights)

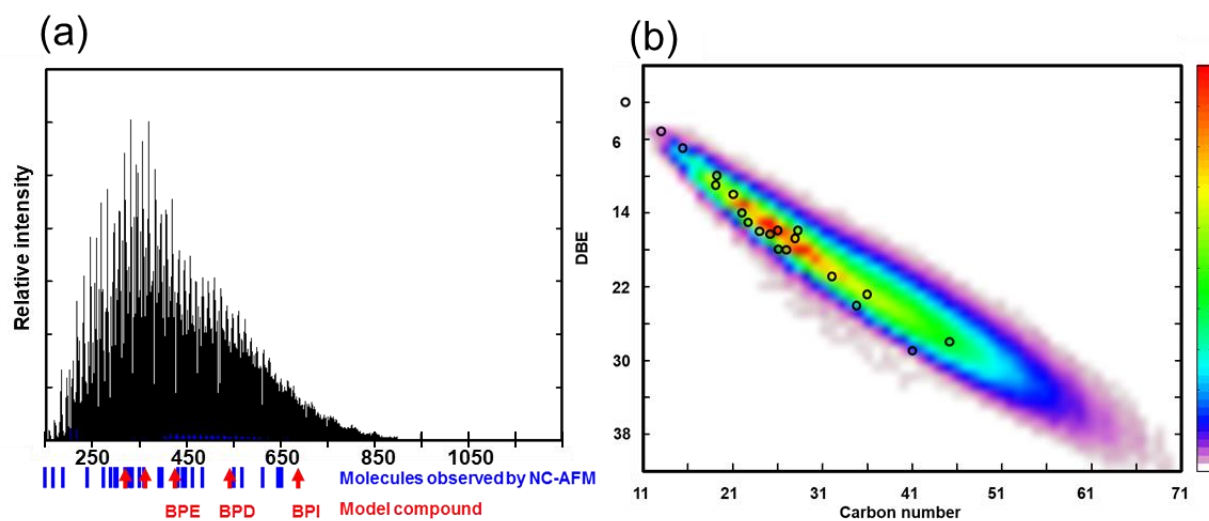
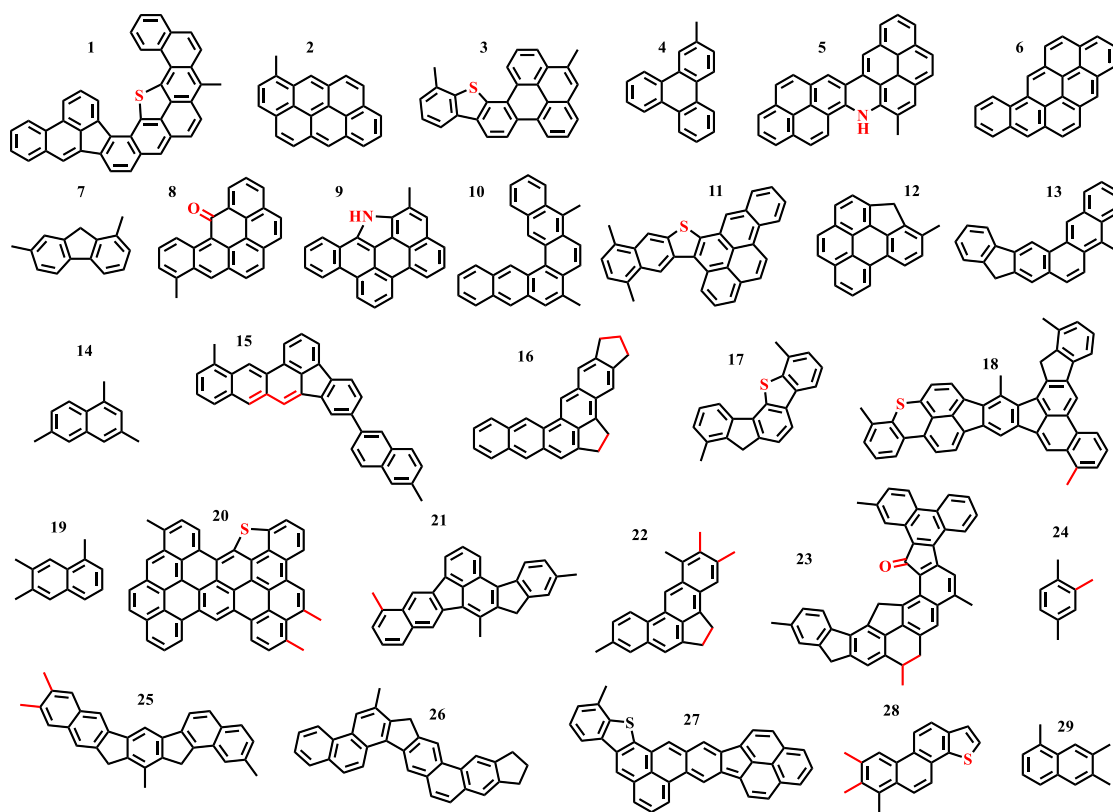


Figure 3. (a) The molecular weights calculated for the AFM-imaged structures is plotted (shown at blue bars at the bottom, with mw of BPE, BPD and BPI as red bars) with the FTICR MS results.

(b) DBE (double bond equivalent) and carbon number obtained by FT ICR MS and AFM imaging (circles indicate individual molecules from AFM imaging).

FT ICR MS analysis of the same sample with atmospheric photoionization (APPI) yielded a molecular weight range of 155 – 896 in **Figure 3a**, which shows clearly that both profiles of molecular weight are remarkably consistent; even the frequencies of observations for the most abundant molecular weights from both methods are consistent. Furthermore, DBE (double bond equivalent) and carbon numbers obtained from the AFM structures are also consistent with the MS results; few assigned structures from AFM images were found outside of the 2D heat map of structures measured from the FTICR-MS data (**Figure 3b**). This consistency is striking since different sampling techniques and detection techniques (molecular ions detected by FT-ICR MS with Atmospheric Pressure Photoionization, and neutral molecules imaged by AFM after flashing heating) were used. We conclude that the AFM imaging results are representative of the overall sample composition.

The AFM imaging results are not always consistent with bulk data, and new learnings can be gleaned from such discrepancies with bulk analysis. For example, a significant number of CH<sub>2</sub> groups in fluorene type molecules (3.5% of total H), revealed by molecular imaging, were expected to be detected by proton NMR at around 3.9 ppm. However, no peak was observed. We hypothesized that slight variations in the chemical environments of CH<sub>2</sub> in the ensemble of molecules broadens this peak below the detection limit. It is expected that some other discrepancies with bulk analyses can be caused by the ambiguous groups in the individual imaging data, and it is necessary to calibrate them with bulk analysis data, such as boiling points, molecular weight, elemental composition and NMR, etc. The established structure-property relationships will help to extrapolate results from limited number of specific structures to ensemble molecules in a bulk

sample, and enable more efficient use of convention analytical tools because imaging a large number of molecules in each sample is neither practical nor necessary.

## **Conclusions**

In this study, we analyzed a series of compounds to understand the effect that the sample preparation conditions have on the structural integrity and representativeness of ensemble molecules in petroleum while molecular imaging with NC-AFM. Especially, we focused on the resistive flash heating method employed to transfer molecules from a bulk sample at room temperature to a highly dilute sample at cryogenic temperatures. This addresses the concern of whether molecules with certain molecular architecture, molecular weight and containing certain bonding types are transferrable or crackable. The results of this study on representative heavy petroleum molecule model compounds confirmed that petroleum molecules within the scope here can be imaged intact and that the imaged data can be representative of the overall sample. We reinforce that determining whether a molecular species with a given mass will fragment or not under the heating used in our experiments is far from trivial. However, for molecules out of the scope of this study (molecular weight larger than 1000) that would likely fragment upon heating, different sample preparation methods can be implemented. An example is the electrospray ionization deposition<sup>29-32</sup> used to demonstrate the deposition of molecules with molecular weights more than 10,000 on a surface.

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### **REFERENCES**

1. Algelt, K. H.; Boduszynski, M. M.; Editors, *Composition and Analysis of Heavy Petroleum Fractions*. [In: *Chem. Ind. (Dekker), 1994; 54*]. Dekker: 1994; p 495 pp.
2. Marshall, A. G.; Rodgers, R. P., *Petroleomics: chemistry of the underworld*. *Proc Natl Acad Sci* **2008**, *105*, 18090-18095.
3. Jaffe, S. B.; Freund, H.; Olmstead, W. N., *Extension of Structure-Oriented Lumping to Vacuum Residua*. *Ind. Eng. Chem. Res.* **2005**, *44*, 9840-9852.

4. Gross, L.; Mohn, F.; Moll, N.; Liljeroth, P.; Meyer, G., The Chemical Structure of a Molecule Resolved by Atomic Force Microscopy. *Science (New York, N.Y.)* **2009**, *325*, 1110-1114.
5. Schuler, B.; Meyer, G.; Pena, D.; Mullins, O. C.; Gross, L., Unraveling the Molecular Structures of Asphaltenes by Atomic Force Microscopy. *Journal of the American Chemical Society* **2015**, *137*, 9870-9876.
6. Schuler, B.; Fatayer, S.; Meyer, G.; Rogel, E.; Moir, M.; Zhang, Y.; Harper, M. R.; Pomerantz, A. E.; Bake, K. D.; Witt, M.; Pena, D.; Kushnerick, J. D.; Mullins, O. C.; Ovalles, C.; van den Berg, F. G. A.; Gross, L., Heavy Oil Based Mixtures of Different Origins and Treatments Studied by Atomic Force Microscopy. *Energy & Fuels* **2017**, *31*, 6856-6861.
7. Beuhler, R. J.; Flanigan, E.; Greene, L. J.; Friedman, L., Proton transfer mass spectrometry of peptides. Rapid heating technique for underivatized peptides containing arginine. *Journal of the American Chemical Society* **1974**, *96*, 3990-3999.
8. Maechling, C. R.; Clemett, S. J.; Engelke, F.; Zare, R. N., Evidence for thermalization of surface-desorbed molecules at heating rates of 108 K/s. *The Journal of Chemical Physics* **1996**, *104*, 8768-8776.
9. Schuler, B.; Collazos, S.; Gross, L.; Meyer, G.; Perez, D.; Guitian, E.; Pena, D., From perylene to a 22-ring aromatic hydrocarbon in one-pot. *Angewandte Chemie, International Edition* **2014**, *53*, 9004-9006.
10. Schuler, B.; Zhang, Y.; Collazos, S.; Fatayer, S.; Meyer, G.; Perez, D.; Guitian, E.; Harper, M. R.; Kushnerick, J. D.; Pena, D.; Gross, L., Characterizing aliphatic moieties in hydrocarbons with atomic force microscopy. *Chem Sci* **2017**, *8*, 2315-2320.

11. Gross, L.; Mohn, F.; Moll, N.; Schuler, B.; Criado, A.; Guitian, E.; Pena, D.; Gourdon, A.; Meyer, G., Bond-Order Discrimination by Atomic Force Microscopy. *Science (New York, N.Y.)* **2012**, *337*, 1326-1329.
12. Giessibl, F. J., High-speed force sensor for force microscopy and profilometry utilizing a quartz tuning fork. *Applied Physics Letters* **1998**, *73*, 3956-3958.
13. Becke, A. D., Density-functional thermochemistry. III. The role of exact exchange. *The Journal of Chemical Physics* **1993**, *98*, 5648-5652.
14. Lee, C.; Yang, W.; Parr, R. G., Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Physical Review B* **1988**, *37*, 785-789.
15. Perdew, J. P.; Burke, K.; Wang, Y., Generalized gradient approximation for the exchange-correlation hole of a many-electron system. *Physical Review B* **1996**, *54*, 16533-16539.
16. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.;

Farkas; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J., Gaussian 09, Revision B.01. Wallingford CT, 2009.

17. Sabbah, H.; Morrow, A. L.; Pomerantz, A. E.; Mullins, O. C.; Tan, X.; Gray, M. R.; Azyat, K.; Tykwinski, R. R.; Zare, R. N., Comparing Laser Desorption/Laser Ionization Mass Spectra of Asphaltenes and Model Compounds. *Energy & Fuels* **2010**, *24*, 3589-3594.

18. Freund, H.; Matturro, M. G.; Olmstead, W. N.; Reynolds, R. P.; Upton, T. H., Anomalous side-chain cleavage in alkylaromatic thermolysis. *Energy & Fuels* **1991**, *5*, 840-846.

19. Sabbah, H.; Morrow, A. L.; Pomerantz, A. E.; Zare, R. N., Evidence for Island Structures as the Dominant Architecture of Asphaltenes. *Energy & Fuels* **2011**, *25*, 1597-1604.

20. Boduszynski, M. M.; McKay, J. F.; Latham, D. R., Asphaltenes, where are you? *Asphalt Paving Technol.* **1980**, *49*, 123-143.

21. Pomerantz, A. E.; Hammond, M. R.; Morrow, A. L.; Mullins, O. C.; Zare, R. N., Two-Step Laser Mass Spectrometry of Asphaltenes. *Journal of the American Chemical Society* **2008**, *130*, 7216-7217.

22. Mullins, O. C.; Joshi, N. B.; Groenzin, H.; Daigle, T.; Crowell, C.; Joseph, M. T.; Jamaluddin, A., Linearity of Near-Infrared Spectra of Alkanes. *Applied Spectroscopy* **2000**, *54*, 624-629.

23. Grill, L.; Rieder, K.-H.; Moresco, F.; Jimenez-Bueno, G.; Wang, C.; Rapenne, G.; Joachim, C., Imaging of a molecular wheelbarrow by scanning tunneling microscopy. *Surface Science* **2005**, *584*, L153-L158.

24. Blanksby, S. J.; Ellison, G. B., Bond Dissociation Energies of Organic Molecules. *Acc. Chem. Res.* **2003**, *36*.
25. Pavlicek, N.; Mistry, A.; Majzik, Z.; Moll, N.; Meyer, G.; Fox, D. J.; Gross, L., Synthesis and characterization of triangulene. *Nature Nanotechnology* **2017**, *12*, 308-311.
26. Pavlicek, N.; Schuler, B.; Collazos, S.; Moll, N.; Perez, D.; Guitian, E.; Meyer, G.; Pena, D.; Gross, L., On-surface generation and imaging of arynes by atomic force microscopy. *Nature Chemistry* **2015**, *7*, 623-628.
27. Gross, L.; Mohn, F.; Moll, N.; Meyer, G.; Ebel, R.; Abdel-Mageed Wael, M.; Jaspars, M., Organic structure determination using atomic-resolution scanning probe microscopy. *Nat Chem* **2010**, *2*, 821-825.
28. McKenna, A. M.; Donald, L. J.; Fitzsimmons, J. E.; Juyal, P.; Spicer, V.; Standing, K. G.; Marshall, A. G.; Rodgers, R. P., Heavy Petroleum Composition. 3. Asphaltene Aggregation. *Energy & Fuels* **2013**, *27*, 1246-1256.
29. Rauschenbach, S.; Vogelgesang, R.; Malinowski, N.; Gerlach, J. W.; Benyoucef, M.; Costantini, G.; Deng, Z.; Thontasen, N.; Kern, K., Electrospray Ion Beam Deposition: Soft-Landing and Fragmentation of Functional Molecules at Solid Surfaces. *ACS Nano* **2009**, *3*, 2901-2910.
30. Hamann, C.; Woltmann, R.; Hong, I. P.; Hauptmann, N.; Karan, S.; Berndt, R., Ultrahigh vacuum deposition of organic molecules by electrospray ionization. *Review of Scientific Instruments* **2011**, *82*, 033903/033901-033903/033906.

31. Deng, Z.; Thontasen, N.; Malinowski, N.; Rinke, G.; Harnau, L.; Rauschenbach, S.; Kern, K., A Close Look at Proteins: Submolecular Resolution of Two- and Three-Dimensionally Folded Cytochrome c at Surfaces. *Nano Letters* **2012**, *12*, 2452-2458.

32. Hinaut, A.; Meier, T.; Pawlak, R.; Feund, S.; Johr, R.; Kawai, S.; Glatzel, T.; Decurtins, S.; Mullen, K.; Narita, A.; Liu, S.-X.; Meyer, E., Electrospray deposition of structurally complex molecules revealed by atomic force microscopy. *Nanoscale* **2018**, *10*, 1337-1344.