

# Fluctuating nanofluid environments induce prebiotic RNA formation in water, which in turn catalyzes polyaromatic semiconductor deposition

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## Research Article

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# Abstract

Water plays an ambivalent role in basic and applied sciences: its beneficial non-toxicity and ubiquity are opposed by severe problems for physicochemical processes that are important in research and technology. In the research on the emergence of life, the so-called “water paradox” is one of the long-standing problems. It states that although water is essential to life, key chemical reactions such as the synthesis of RNA are inhibited by it. Current strategies to circumvent this paradox have low prebiotic plausibility when considering the conservative nature of evolution. Here, we report a solution to overcome the paradox in line with evolutionary conservatism. We achieved spontaneous non-enzymatic RNA formation in aqueous environments by harnessing fluctuating nanofluid conditions that alter critical properties of water. These conditions are generated in aqueous particle suspensions of varied composition – a geochemically ubiquitous and thus prebiotic highly plausible environment. We give experimental evidence for synergy between biomolecular assembly and nanofluid effects between suspended particles and demonstrate that this type of abiotic environment allows prebiotic condensation reactions towards RNA synthesis in water under stable, moderate conditions. This is consistent with the principle that evolution builds on existing pathways, as macromolecules in living cells also work with temporal nanofluid water to run biopolymerization. Beyond their high significance for prebiotic chemistry, our findings also open up a pathway to sustainable chemistry approaches in materials science and nanotechnology. We demonstrate this by biomolecular-induced catalysis of monolayer deposition from water-insoluble organic semiconductors under aqueous nanofluid conditions.

# Introduction

Prebiotic chemistry is facing a serious problem in regard to the role of water in abiogenesis: although water is essential for all life as we know it, key biochemical reactions such as the polymerization of nucleotides into RNA are impeded in watery solutions.<sup>1</sup> In aiming to overcome this so-called “water problem” in prebiotic chemistry,<sup>2</sup> several hypotheses have been proposed.<sup>3-6</sup> Among them are concepts of adding condensing agents such as cyanamide, using alternative solvents such as formamide, setting high temperatures of about 160°C, or designing prebiotic scenarios based on wet/dry cycles. However, when appraising the plausibility of such concepts and scenarios, some general weaknesses appear.<sup>4</sup> A key aspect is that all known life manages the water problem within a stable environment full of water and does not rely on physical conditions and chemical substances proposed in these concepts. This aspect is of particular relevance regarding evolutionary conservatism – the principle that evolution builds on existing pathways.<sup>7</sup> In this context, the principle indicates that the same physicochemical effects were involved in the abiotic origin of biopolymers, as is now being tapped by living systems via complex enzymes. Here we demonstrate how the water paradox in prebiotic chemistry is solvable without facing these weaknesses by considering nanofluid effects on water. According to our results, abiotic polymerization of nucleotides into RNA can occur within aqueous dispersions of polyaromatic hydrocarbons and inorganic minerals in the absence of enzymes or additives. We present the synthesis of various amounts of RNA depending on variations of the experimental parameters and propose an

explanatory model based on nonclassical effects on water when temporarily confined by suspended particles. The model describes how such fluctuating nanoconfinements solve the water paradox by altering crucial physicochemical properties of water and how evolutionary conservatism can link these natural nanofluid reaction vessels to enzymatic biopolymerization in biological cells.

Living cells contain an intracellular aqueous fluid that is crowded with large, complex biomolecules. In this environment, virtually all water exists as interfacial water.<sup>8</sup> When viewing this dense mixture from the perspective of materials science, it is describable as an aqueous suspension of highly concentrated nanoparticles. In the vicinity of such particles, various nanofluid phenomena emerge in interfacial and nanoconfined water.<sup>9,10</sup> Consequently, such water differs significantly compared to bulk water in terms of properties such as reactivity, H-bonding network dynamics, density, dielectric constant, or the quantum state of protons.<sup>11-13</sup> From a geochemical point of view, aqueous suspensions of mineral particles of micro- and nanoscopic size can be regarded as a comparable environment that generates nanofluid effects. Inspired by this similarity, we designed experiments to test the potential of nanofluid environments within aqueous suspensions of particles for inducing key biochemical reactions in a possibly prebiotic context. As the abiotic synthesis of RNA in water is a common goal of prebiotic chemistry and synthetic biology,<sup>14,15</sup> we chose the polymerization of nucleotides into RNA as an example reaction. The focus was set on the formation of a pure adenosine-based RNA (poly-A-RNA) since adenosine monophosphate (AMP) is the most common nucleotide in living cells.<sup>16</sup> Furthermore, poly-A-RNAs are common RNAs in cells in the form of poly-A-tails of messenger RNAs during the process of protein biosynthesis. Thus, we first prepared samples that contained dissolved AMP. To create a nanofluid environment for AMP solutions, we selected quinacridone (QAC) as an example of polyaromatic heterocycle particles and graphite as an inorganic suspended particle species, as both compounds have been well characterized in terms of inducing nanofluid phenomena in aqueous suspensions.<sup>17</sup>

## Results And Discussion

### Fluorometric poly-A-RNA concentration determination

Figure 1a shows the results of fluorometric RNA concentration measurements of such samples. Ethanol precipitation to isolate possible poly-A-RNAs of 2-10 nt lengths and isopropanol precipitation to isolate such RNAs longer than 10 nt were applied. The results of both precipitation methods reveal that poly-A-RNA strands have formed in significant amounts regarding the negative controls (Fig. 1c) and that oligonucleotides longer than 10 nt lengths are abundant in such samples. After having incubated a sample for over four weeks at room temperature, the presence of poly-A-RNA was still detected in a similar concentration (2.53 ng/ $\mu$ l) with respect to the comparable suspension sample with the standard incubation procedure overnight ((2.38 ng/ $\mu$ l, Fig. 1a).

## RT-qPCR analysis

To cross-check the fluorometric analysis approach with a totally independent but also highly specific method, we performed quantitative polymerase chain reaction after reverse transcription (RT-qPCR). The results (Fig. 2a) show that the signal of the sample exceeds the background fluorescence after 7.57 cycles. This indicates a high yield (103-fold) of input RNA in comparison to the respective signal of the positive control miRNA (14.25 cycles). To obtain more information about the length of the formed RNA, we performed a comparative melting curve analysis. This analysis included the sample RNA and a positive control miRNA with a length of 23 nt. The comparison reveals that the medium melting point of the sample is at a higher temperature than the positive control (Fig. 2b). These results suggest that the sample contains created oligonucleotides with lengths equal to or longer than 23 nt.

## Identifying factors that determine abiotically induced polymerization

To identify substantial factors that lead to the observed abiotic polymerization, we first analyzed the possible role of  $\pi$ -stacking of nucleoside monophosphates (NMPs), as stacking has been suggested to be an important factor for RNA polymerization in terms of bringing the monomers in close contact.<sup>18</sup> To perform this analysis, we extended the fluorometric analysis of ethanol precipitated AMP-based samples to CMP- and UMP-based samples and compared the relationship of the different RNA concentration results with the relationship of respective published stacking equilibrium constants and stacking free energies of NMPs. The comparison reveals that the detected relative concentration of poly-A-RNA is about four times higher than that of poly-C-RNA and poly-U-RNA (Fig. 1a) and that this order (poly-A-RNA >> poly-C-RNA ~ poly-U-RNA) correlates to the order of the respective stacking equilibrium constants and stacking abilities derived from the stacking free energy profiles of NMPs.<sup>19</sup> This correlation suggests that in particle suspensions, stacking and polymerization of nucleotides are linked. Therefore, enhancing the stacking ability of a nucleotide should result in a higher RNA concentration. To test this hypothesis, we selected UMP due to its low self-stacking ability and its comparatively low poly-U-RNA formation and added the amino acid arginine to the sample. Arginine is known for its high stacking ability with aromatic groups.<sup>20</sup> Fluorometric analysis of UMP/arginine-based samples revealed that arginine increased the formation of poly-U-RNA by more than 100% (Fig. 1a). This observation supports the necessity of  $\pi$ -stacking for polymerization in aqueous suspensions and points to a possibly prebiotically relevant kind of interplay between amino acids and nucleic acids.

However, base stacking cannot be the key factor for RNA formation in the described samples. This becomes evident when taking the water paradox into account and when considering the result of a negative control based on an AMP solution without suspended particles (Fig. 1c): although AMP has the highest self-stacking constant among all NMPs<sup>19</sup> and is the only NMP with the ability to self-associate in indefinite stacks,<sup>21</sup> the negative control shows that in comparison to the poly-A-RNA concentrations reported above, only minute amounts can be found when the sample contains no added particles. This result suggests that there is a key factor in promoting both stacking and polymerization that is closely linked to the particle suspension nature of the samples.

It is known that reducing the dielectric constant of water favors stacking,<sup>21</sup> and reducing the activity of water (e.g. by adding alternative solvents, inducing wet/dry cycles, or intercalation) promotes polymerization.<sup>3,22,23</sup> As nanofluid phenomena emerging in temporal nanoconfined water can reduce both properties simultaneously<sup>12,24</sup> and watery suspensions of particles give rise to such phenomena,<sup>9</sup> we infer that the occurrence of nanofluid phenomena in our samples is the key factor for the observed nucleotide polymerization. This implies that the enhancement of nanofluid effects should correlate with an increase in polymerization reactions.

### **Comparing the extent of nanofluid effects to polymerization efficiency**

Nanoconfining environments change the behavior of water, especially in terms of its hydrogen bond network dynamics,<sup>12</sup> which, in turn, affects the thermodynamic property of water activity<sup>24</sup>. The anomalous behavior of nanoconfined water results from a highly complex interplay of various nanofluid phenomena and forces that are related to, for example, the surface energy and size of the confining boundaries, shear, molecular structure, electrical double layer, and fluctuations of general order parameter.<sup>9,25</sup> To cope with this high complexity when assessing a possible synergy between nanofluid phenomena and polymerization, we chose an experimental approach that allows us to focus on a single, quantifiable effect. This approach uses the effect of organic solid/solid wetting deposition (OSWD) as a probe. OSWD is the final result of a network of various nanofluid phenomena on confined water between suspended organic crystals, including double-layer forces, Casimir-like fluctuation-induced forces, and dewetting-induced hydrophobic collapse.<sup>17</sup> It manifests as the adsorption and self-assembly of insoluble polyaromatic heterocycles at solid/solid interfaces and thus is quantifiable via surface coverage determination.

We quantified the efficiency of OSWD as a function of different biomolecules dissolved in watery suspensions of particles of the organic semiconductor QAC. For quantification, we measured the surface coverage of graphite with QAC monolayers via scanning tunneling microscopy (see Supplementary Fig. 1, Fig. 2). The results (Fig. 3) reveal that adding AMP to the particle suspension enhances the coverage with very high statistical significance ( $p < 0.0001$ ) in relation to QAC suspensions based on pure water (w/o, Fig. 3). A significant increase in coverage ( $p < 0.01$ ) also occurred when using arginine as the added biomolecule. By contrast, coverages found in samples based on CMP or UMP are statistically nearly identical and significantly lower with respect to the AMP- and arginine-based samples. Using a mixture of different NMPs leads to no significant increase in coverage but results in some very high single coverage measurements regarding the CMP and UMP samples (NMPs, dot plot, Fig. 3), possibly caused by clusters of the AMP fraction of the mixture. The comparison of these OSWD-induced coverages with RNA quantities (Fig. 1a) indicates that relative differences in the extent of nanofluid phenomena between the samples correlate with relative differences in detected RNA quantities. This supports the hypothesis of synergy between nanoconfinement effects and nucleotide polymerization in aqueous particle suspensions. We thus propose that the emergence of nanofluid effects on confined water is the key

factor in the promotion of both stacking and polymerization of nucleotides within aqueous suspensions of particles.

### **Assessing the generalizability of suspension-induced polymerization**

Thus far, we used QAC/graphite particle systems to ensure comparability of the results from different experimental approaches. To test whether the formation of RNA in such an environment is generalisable, we measured samples prepared by the same protocol but containing particles based on other compounds. As inorganic substitutes, we chose magnetite and silica as geologically widespread compounds and selected the organic compound anthraquinone due to its abundance in carbonaceous meteorites.<sup>26</sup> Fluorometric measurements of partly or fully substituted samples show poly-A-RNA concentrations that are similar to or substantially higher than the comparable output of the QAC/graphite model system (Fig. 1b). This indicates that the abiotic formation of RNA within nanofluid environments of aqueous suspensions is of general nature. Our results also imply that the output of RNA can be increased to higher concentrations by identifying appropriate particle suspension systems. The influence of the particle species on the RNA concentration, as indicated by Fig. 1b (including results from inert polyethylene particles as a comparison), is consistent with the fact that the anomalous behavior of nanoconfined water is partly determined by the characteristics of the confining surfaces.<sup>12</sup>

### **Dynamic molecular mechanics calculations**

For nanofluid effects to become dominant, the confining surfaces must approach, at a conservative estimate, within the range of 10 nm and below.<sup>12</sup> To evaluate whether stacking of nucleotides within such nanoconfinements is possible in a sufficiently stable and ordered way to prime polymerization, we performed dynamic molecular mechanics calculations. For performing these calculations, we modeled a stack of 12 AMPs as an example system referring to the observation that polymerized AMPs of more than 10 nt length were abundant in our samples. We arranged this stack in parallel between two nanoscale separated crystals of a QAC/graphite system and added water to the confinement. Figure 4a shows the condition of the stack at the end of a dynamic simulation modeled with a confinement gap size of about 4 nm. This condition implies that the nucleotide stack remained arranged in such confinement when surrounded by water (see also Supplementary Video 1). Comparative calculations suggest that such stacks are deformed, but stable even when the density of water – which can be lower in nanoconfined conditions<sup>12</sup> – is decreased to approximately 60% (Fig. 4b, Supplementary Video 2). It requires a reduction of the gap size to about 3 nm and below to finally destabilize a stack due to increased interactions with confining surfaces (Supplementary Fig. 3). In sum, these simulations indicate that it is feasible to assume that nucleotides can associate in stable stacks within nanoconfinement gap sizes well below 10 nm for priming polymerization.

### **Thermodynamic considerations**

Nucleotide polymerization into RNA is a water-releasing condensation reaction that faces a thermodynamic barrier as the change in the Gibbs free energy of such a reaction is positive.

Consequently, nucleotide polymerization in water is a thermodynamically uphill reaction that is extremely inefficient to occur spontaneously under ambient conditions. However, if the entropic part ( $\Delta S$ ) of the Gibbs free energy change ( $\Delta G = \Delta H - T \Delta S$ ) becomes very positive, the reaction can become exergonic ( $\Delta G < 0$ ) and thus favorable. As the entropic part is positive when the activity of water is low,<sup>22</sup> the thermodynamic barrier can be overcome by reducing water activity.<sup>27</sup> Against this background, we propose that our observed abiotic formation of RNA within aqueous particle suspensions can be explained by the rise of anomalous properties of water when getting temporarily confined between suspended particles: nanoscale confinements change, among others, the vapor pressure<sup>28</sup> and hydrogen-bond network dynamics<sup>12</sup> of water. This can reduce water activity<sup>24,28</sup> as a function of gap size and the characteristics of the confining surfaces. We suggest that in comparison with other ways to circumvent the thermodynamic barrier of such condensation reactions, the exergonic impact of nanofluid phenomena in aqueous particle suspensions on RNA polymerization and stabilization is of high relevance for prebiotic plausibility, as it does not require nonphysiological conditions such as temperatures well above 100 °C, alternative solvents, or wet/dry cycles.

## Conclusion

Our results indicate that abiotic temporal nanoconfinements of water within aqueous particle suspensions serve as nanoscopic, flexible reaction vessels for prebiotic, nonenzymatic RNA formation. The findings can solve the water paradox in such a way that nanofluid effects in aqueous particle suspensions open up an abiotic route to biopolymerization and polymer stabilisation under chemical and thermodynamic conditions that are also prevalent within the crowded intracellular environment of living cells. The fact that polymerase enzymes also form temporal nanoconfined water clusters inside their active site<sup>29,30</sup> implies that the same physicochemical effects are tapped for nucleotide condensation in water both by biochemical pathways and our reported abiotic route. These aspects indicate that our model is consistent with evolutionary conservatism stretching back to the era of prebiotic chemical evolution and the origin of cellular life.<sup>31</sup> The consistency is further supported by the fact that water is not trapped by nanoconfinements within the polymerase core but can exchange with the surrounding intracellular fluid<sup>30</sup> – a situation that also exists in abiotic nanoconfinements of water emerging temporarily between approaching crystal particles in aqueous suspensions. Our experimental finding that under these conditions an amino acid catalyses the abiotic polymerization of nucleotides supports first indications of the role of minerals for the origin of cooperation between amino acids and nucleotides<sup>32</sup> evolved to the interdependent synthesis of proteins and nucleic acids in living cells.<sup>33</sup> Abiotic RNA polymerization under nanofluid conditions in aqueous particle suspensions does not depend on specific mineralogical and geological environments: now as then, in the prebiotic world, watery suspensions of micro- and nanoparticles are virtually ubiquitous.<sup>34</sup> They exist, for example, in the form of sediments with pore water,<sup>35</sup> hydrothermal vent fluids containing precipitated inorganic<sup>36,37</sup> and polyaromatic<sup>38</sup> particles, or crowded, dispersed aggregates inside water-filled cracks in the crust of the earth,<sup>39,40</sup> and possibly of icy moons such as Enceladus.<sup>41</sup>

Beyond the discussed importance of our findings for prebiotic chemistry and theories on the transition from geochemistry to biochemistry in the course of life's emergence, the results are also highly relevant for the development of sustainable chemistry approaches in applied sciences: the detected substantial efficiency increase of OSWD-induced monolayer formation of widely used industrial organic semiconductors such as QAC (Fig. 3) or phthalocyanine (Supplementary Fig. 4) via biomolecules with a prominent stacking capability such as AMP and arginine provides a systematic path to catalyze the deposition of water-insoluble organic semiconductors on 2D materials via nanofluid water. This insight is substantial for the development of green chemistry approaches to chemical surface engineering, band-gap engineering, and low-dimensional crystal engineering in materials science and nanotechnology.

## Methods

### Compounds

Adenosine-5'-monophosphate (AMP, Sigma, disodium salt, CAS-Nr. 61-19-8); Uridine-5'-monophosphate (UMP, Alfa Aesar, disodium salt, CAS-Nr. 58-97-9); Cytidine-5'-monophosphate (CMP, Alfa Aesar, disodium salt, CAS-Nr. 6757-06-8); Guanosine-5'-monophosphate (GMP, Alfa Aesar, disodium salt, CAS-Nr. 85-32-5); L-Arginine (Alfa Aesar, CAS-Nr. 74-79-3); Quinacridone (Clariant GmbH, CAS-Nr. 1047-16-1); Anthraquinone (Fluka, CAS-Nr. 84-65-1); Phthalocyanin ( $\beta$ -form, Sigma-Aldrich, CAS-Nr. 574-93-6); carbon nanopowder (<50 nm (TEM), Sigma Aldrich, CAS-Nr. 7440-44-0); Carbon (mesoporous, Sigma Aldrich, CAS-Nr. 1333-86-4); Iron (II, III) oxide (Sigma Aldrich, CAS-Nr. 1317-61-9); Silicon dioxide (-325mesh, Sigma Aldrich, CAS-Nr. 60676-86-0); Polyethylene (<400 micron, Alfa Aesar, CAS-Nr. 9002-88-4).

### Suspension preparation

Three milliliter of aqueous suspension was made of each 0.1 g/ml inorganic substrate (e.g., graphite powder) and/or organic pigment with a total nucleoside monophosphate concentration of 50 mM. Samples were incubated overnight at 60°C while mixed horizontally at 300 rpm, to avoid sedimentation of substrates and pigment. After incubation, samples were centrifuged at 8000x g for 1 min at room temperature (RT). The supernatant was transferred to a new collection tube.

### Precipitation

Precipitation was carried out with 0.2 M NaCl and 3.5 volumes of EtOH for isolation of short RNAs (2–10 nt), and with 0.3 M NaOAc (pH 5.2) and 0.7 volumes of isopropanol for isolation of longer RNAs (> 10 nt). After the addition of the appropriate amount of salt solution and alcohol, samples were mixed by inverting the tubes 5 times, and precipitation reactions were incubated overnight at -20°C. Afterwards the samples were subsequently centrifuged at 14.000x g, for 1 hour at room temperature. The supernatant was discarded, leaving approximately 20  $\mu$ l of it inside the reaction tube, in addition to any formed gel

pellet. Formed gel pellets were dried at 37°C for 20 min and resuspended in an appropriate amount of H<sub>2</sub>O dest.

### **Concentration measurements**

Concentrations of miRNA suspensions were measured using a Qubit® 3 Fluorometer (Invitrogen™),<sup>42</sup> the Qubit® microRNA Assay Kit (Invitrogen™) or the Qubit® RNA HS Assay Kit (Invitrogen™) due to its high specificity and reliability.<sup>43</sup> Concentrations were calculated by the chosen miRNA or RNA program of the Qubit® 3 Fluorometer. Standard curves and samples were prepared following the manuals.<sup>44</sup> Concentrations were calculated and normalized regarding the respective volume of each sample.

### **Quantitative RT-qPCR**

For RT-qPCR, selected samples were reverse transcribed using the TaqMan™ Advanced miRNA cDNA Synthesis Kit (Thermo Fisher) following the manual. As a positive control, the synthetic miRNA hsa-miR-134-3p (5'-phosphorylated) (eurofins) with an oligonucleotide length of 23 nt was used. As a negative control nuclease-free water was used. For qPCR, the Master Mix reaction was composed of 10 µl QuantiTect SYBR® Green (Qiagen), 2 µl amplification primer mix from the TaqMan™ Advanced miRNA cDNA Synthesis Kit (Thermo Fisher), and 8 µl of diluted sample. qPCR was ran on a LightCycler® 480 Instrument II with the following settings: 15min 95°C, 40x: 15s 94°C – 30s 60°C – 30s 72°C – Single Data Acquisition, 72°C 2 min, Melting curve.

### **Scanning tunneling microscopy**

For scanning tunneling microscopy (STM) investigations aqueous suspensions were prepared of each 2% w/w quinacridone pigment and a nucleotide monophosphate or amino acid final total concentration of 25 mM, if required. Suspensions were drop cast onto graphite substrates to induce OSWD16 and dried overnight. The samples were subsequently covered with dodecane to achieve a defined, stable environment between the STM tip and substrate. Each sample was prepared twice. From each replicate, 15 images of 200 nm x 200 nm size were generated from 3 different spots. Images were acquired with a self-built STM combined with an SPM 100 control system supplied by RHK Technology. The scan settings were: bias = 1 V, tunnel current = 300 pA, and line time = 50 ms. Further, the voltage pulses used to improve the scan quality were applied within the range of 4.3 V to 10 V. All STM measurements were performed under ambient conditions.

### **Surface coverage determination**

To determine coverage, meaning how much area of graphite is covered by supramolecular adsorbate structures (Supplementary Fig. 1), black/white histograms were created. For this purpose, depicted adsorbate structures were masked out in the STM image (white) while setting the background (graphite without adsorbate structures) to black (Supplementary Fig. 2). Black/white histogram values were calculated using “histogram” within the “analyze” menu of the software ImageJ. The mean values given

in the histogram data box were then converted to percentages of the graphite area covered with adsorbate structures, whereby a histogram value of 0 means 0% of the graphite surface is covered with adsorbate and a histogram value of 255 means 100% of the graphite surface is covered. Note that AMP itself does not form monolayer on graphite due to its 3D-structure. For statistical analysis of the coverage results, a t-test with Welch's corrections was used since the samples show unequal distribution variance. The calculations were performed using Excel, giving the results of a two sided unequal variance t-test.

## Computer simulations

All molecular mechanics calculations were performed with the Materials Studio package (Accelrys). The force field used was Dreiding,<sup>45</sup> where partial charges of atoms within the molecule are calculated with the Gasteiger method.<sup>46</sup> Geometry optimization calculations were calculated where convergence tolerance regarding energy was  $1.0e-4$  kcal/mol, force was  $0.005$  kcal/mol/Å and displacement was  $5.0e-5$  Å. The Smart algorithm was used which is a concatenation of steepest descent, Newton-Raphson and quasi-Newton methods, to obtain better behavior for the different stages of downstream minimization. Dynamic calculations were performed for an NVE ensemble with a temperature of 298 K, random values assigned for the initial velocities of the atoms and a time step of 0.1 fs for the integration algorithm.

A chain of 12 AMP molecules was constructed starting with building an AMP dimer. The dimer itself was built by duplicating an AMP and performing an 180° rotation related to the axis lying in the plane of the adenine molecule and passing through the centres of the hexagon and pentagon. Afterwards, the rotated AMP was translated normal to the plane of the adenine by a distance of 3.5 Å. This dimer was stacked 6 times in a row such that all planes of the adenine molecules were parallel each by a distance of 3.5 Å. This artificially constructed model was finally optimized related to geometry.

The entire system is modeled within a supercell containing two graphene layers, on top at some distance a chain of AMP molecules and finally two layers of a QAC crystal above the AMP chain. The entire system was filled with water molecules at different concentrations. The initial position of the AMP chain related to the graphene layers and the QAC crystal layers is stated below for the different calculations performed. The vertical dimension of the supercell has been chosen to be safe so that an interaction of the graphene layers and the QAC crystal layers can be neglected (gap is 43 Å). The rectangular in-plane dimensions of the supercell have been determined to minimize the distortion of the periodic structure of the QAC crystal layers. The deviation of the unit cell vectors of the QAC layers is almost zero in one direction (0.1%) but quite large in the other direction (5.6%). However, because the outer layer of the QAC crystal is always kept fixed, the adjacent free movable QAC layer was observed to be stable throughout all simulations. This observation was the reason why this significant deviation was accepted for all calculations. The graphene layers are not distorted with regard to the optimal unit cell dimensions. The dimensions of the supercell are: plane x, y: 80.956 Å, 56.58 Å, and z: 103.4 Å.

## Declarations

## ASSOCIATED CONTENT

### Supporting Information

The following files are available free of charge.

Figure S1, showing STM images of a graphite surface after OSD processing; Figure S2, giving an example of a black/white conversion of STM images for coverage analysis; Figure S3, showing the results of Dynamic force field calculations of an AMP stack within nanoconfined water.

Movie files: Movie M1, showing the result of a simulation of an AMP stack within a confinement filled with water; Movie M2, showing the result of a simulation similar to M1, but with a water density reduced to approximately 60% (AVI).

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### Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. A.G.H. designed and performed experiments and data analysis, T.M. designed and performed computer simulations, F.T. conceived the project and directed the research.

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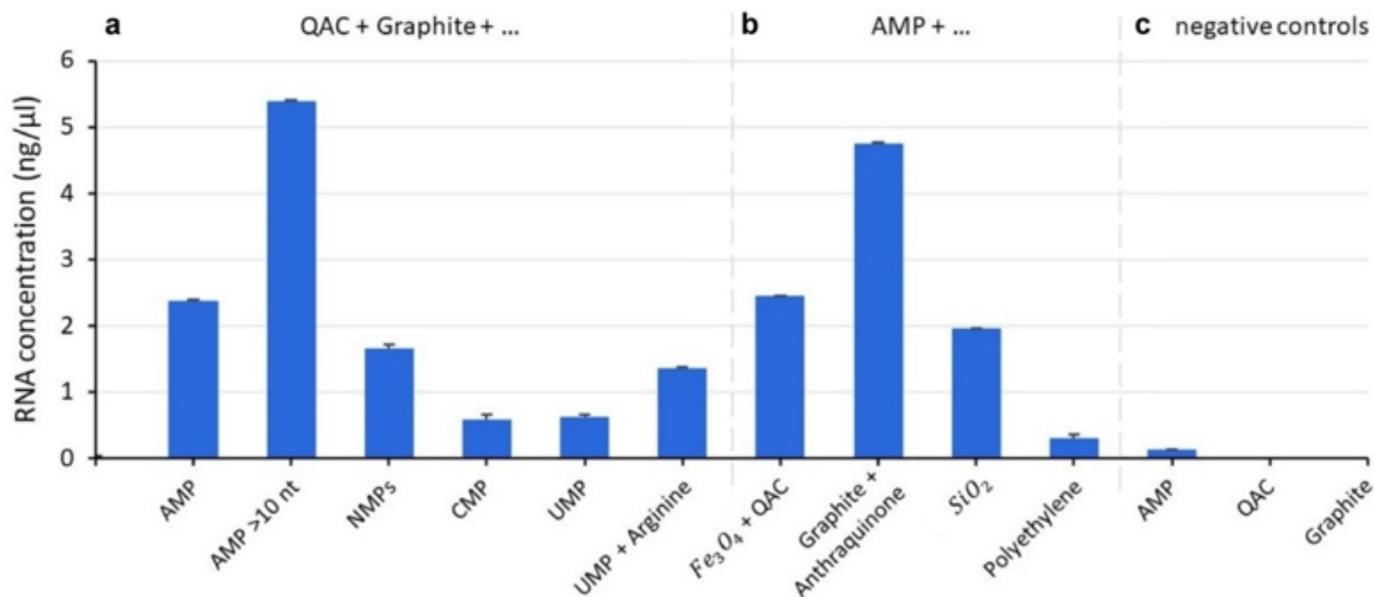
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## Figures

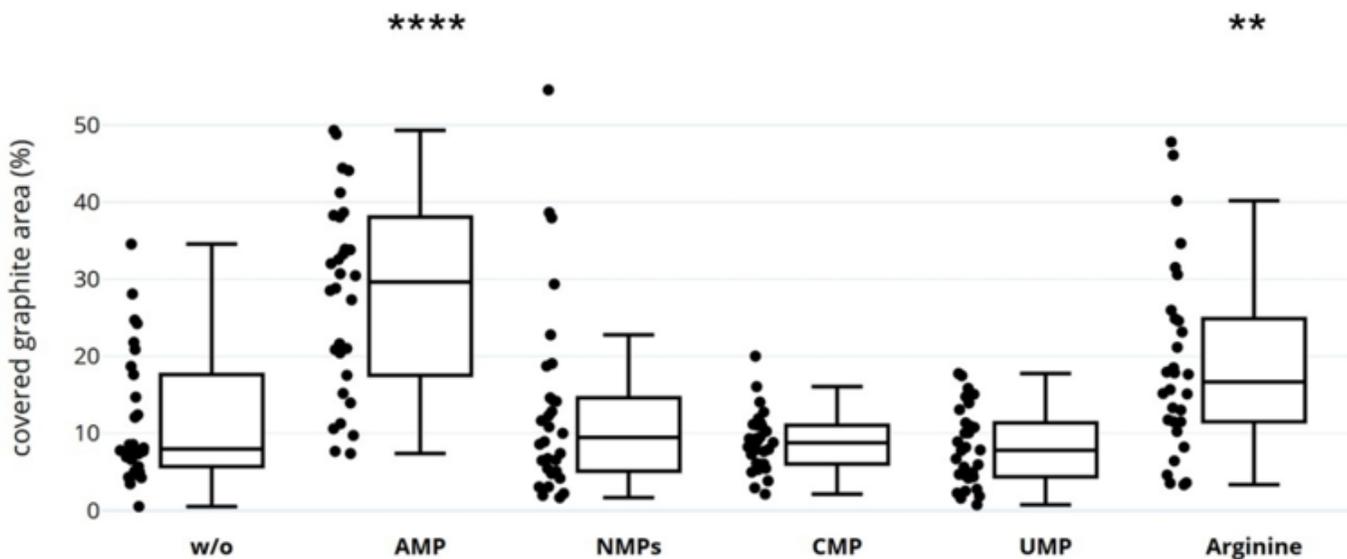


**Figure 1**

Fluorometric results of RNA concentration measurements in aqueous particle suspensions. Displayed are the mean values of three measurement replicates and standard deviations. If not differently indicated, nucleotide suspensions had a final concentration of 50 mM. RNA isolation was performed from aqueous suspensions of particles containing dissolved biomolecules. **a**, Detected RNA concentrations in samples based on QAC + graphite suspensions: AMP: 2.38 ng/μl; AMP > 10 nt: 5.4 ng/μl; mixture of NMPs (AMP, UMP, GMP, CMP, 12.5 mM each): 1.66 ng/μl; CMP: 0.59 ng/μl; UMP: 0.62 ng/μl; mixture of UMP and arginine: 1.36 ng/μl. **b**, Detected poly-A-RNA concentrations in suspensions based on: Fe<sub>3</sub>O<sub>4</sub>/QAC: 2.45 ng/μl; graphite/anthraquinone: 4.76 ng/μl; SiO<sub>2</sub>: 1.96 ng/μl; polyethylene: 0.3 ng/μl. **c**, Results of the negative controls: aqueous samples containing AMP but without suspended particles: 0.14 ng/μl; pure aqueous samples only containing QAC or graphite: both underneath the detection level.

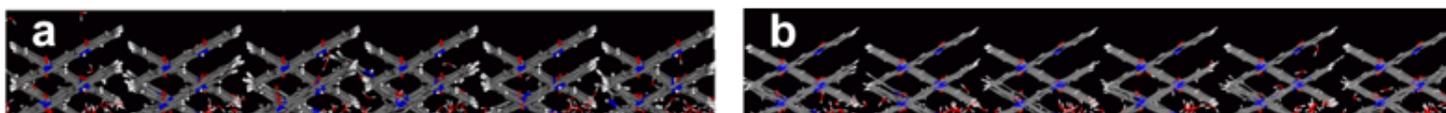
**Figure 2**

Quantitative PCR after reverse transcription (RT-qPCR) applied to an aqueous particle suspension with dissolved AMP. Yellow: negative control; green: positive control (synthetic miRNA hsa-miR-134-3p (5'-phosphorylated)); black: sample (precipitation from sample "QAC + Graphite + AMP", Fig. 1a). **a**, Fluorescent signals of qPCR. The signals exceed the background fluorescence (yellow) after 7.57 cycles (sample) and 14.25 cycles (positive control). **b**, Melting curves. Medium melting point: positive control (green): 80.53 °C, sample (black): 81.31 °C.



**Figure 3**

OSWD efficiency as a function of biomolecular species added to aqueous particle suspensions. The box plots and distributions of values are based on results obtained by STM. The statistical analysis of the results reveals the coverage of graphite surfaces with organic semiconductor QAC monolayer. The adsorbate forms after graphite has been brought into contact with a watery suspension of QAC particles that either contains dissolved biomolecules (AMP, NMPs, CMP, UMP, arginine) or no biomolecules (w/o). Testing: t-test with Welch's correction; n=30; \*\*: p<0.01; \*\*\*\*: p< 0.0001.



**Figure 4**

Dynamic force field calculation of a nucleotide stack in nanoconfined water. The system is modelled within a supercell containing two graphene layers (bottom), two layers of a QAC crystal (top) and a stack

of 12 AMP molecules placed between the confining surfaces. Vertical dimension of the gap: 43 Å. a, Condition of the stack after a simulated time span of 50 ps. The confinement is filled with water molecules. b, Condition of the stack after a simulated time span of 60 ps and a water density of approximately 60%.

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