Questioning claims of monitoring Michael-addition-reaction at the single-molecule level

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In this work Yang *et al.* [1] claim that an enantioselective Michael addition reaction with a barrier of 16 kcal/mol occurs at the single molecule level in frozen solvent by measuring fluctuations in current flowing across graphene based molecular devices. The article, however, contains major scientific errors that undermine their conclusions. We highlight issues with the fabrication of the devices, a lack of characterisation, discrepancies between theory and experiment, unreliable Inelastic Electron Tunneling Spectra (IETS) and a perceived misinterpretation of noise as evidence of reaction.

The Fabrication of the Devices Leaves Many Unknowns

The experiments described in the work of Yang *et al.* are based on the fabrication of graphene electrodes separated by a small distance, which should allow bridging by a single molecule. The electron-beam fabrication methods are not accurate enough to produce the gap distance controllably. Although Yang *et al.* claim that the graphene electrodes are terminated by carboxyl groups, this has not been demonstrated experimentally. No independent verification of the molecular bridging geometry is provided. Note that the six phenyl groups of the bridging molecule could also bind by pi-stacking on top of the graphene, leaving room for many different binding configurations and none of the experiments described exclude such alternative binding.

The Magnitude of Measured Current: Eight Orders of Magnitude Missing

When synthesis is claimed to occur one molecule at a time, it may not be possible to characterize the products by standard techniques such as nuclear magnetic resonance (NMR), high-performance liquid chromatography (HPLC) or high resolution mass spectroscopy (HRMS). It is, however, possible to measure a characteristic single-molecule conductance (or current) and show that the same is observed with the *ex-situ* synthesized and characterized product. This minimal level of verification, which is well documented in the literature [2, 3, 4], has not been followed in the work of Yang *et al.* Instead, the authors choose to confirm that the measured currents can be attributed to single-molecule junctions using density functional theory (DFT) based calculations. However, comparing Figure 2b and Supplementary Figure 15, the calculated currents, following the Landauer-Buttiker formalism (Eq. 1), are $10^7 - 10^8$ times smaller than those measured in the experiment.

$$I = \frac{2e}{h}\int_{-\infty}^{\infty} dE\ T(E)(f_L(E - eV/2) - f_R(E + eV/2)) \simeq \frac{2e^2V}{h}T_{avg}(E_F); \qquad (1)$$

Specifically, the average transmission ($T_{avg}(E_F)$) around the Fermi energy in Supplementary Figure 15 is $\sim 10^{-10}$. Using known values for the charge of an electron (e), Planck's constant (h), and voltage $V = 300$ mV, and assuming zero temperature (to set the left and right Fermi-Dirac distribution functions (f_L, f_R) to 1 within the bias window and 0 outside), we obtain a theoretical current of $\sim 2.6 \times 10^{-15}$ A, much smaller than the measured current of 2.5×10^{-7} A in Figure 2A. Note that the calculated current reported in Figure 2b, must have been obtained from the DFT results by 'correcting' for a factor 10^8, without procedure.

Although DFT calculations have well-known errors that are inherent to the method, these errors result in the theory *overestimating* currents by one or two orders of magnitude [5, 6, 7, 8]. An *underestimation*, and especially by more than eight orders of magnitude, cannot be attributed simply to DFT-errors. Thus, the currents in the 50-300 nA range at a bias ~ 0.3 V measured across a hexa-phenyl derivative cannot be attributed to that of a single-molecule junction.

IETS Spectroscopy: Inconsistent with Experimental Resolution

The second piece of evidence for the bridging of the graphene electrodes by this particular single molecule is given by the recorded Inelastic Electron Tunneling Spectra (IETS), shown in Supplementary Figure 8. The information in the caption of this figure is not sufficiently detailed to establish the experimental protocols. Typically, it is very hard to obtain reproducible spectra for IETS, and the recording of IETS spectra requires long averaging times. Seeing that the bias voltage is ramped up to voltages above 0.4 V, the switching of the molecule between the various states should prohibit the recording of stable spectra.

However, the key factor for evaluating the reliability of these data is the modulation voltage used for recording the spectra; this is given as $V_{\mathrm{mod}} = 21.2$ mV. This amplitude limits the resolution of the spectral peaks to $1.7V_{\mathrm{mod}} = 36$ mV (FWHM) [9]. The two most prominent peaks in Supplementary Figure 8a have a width of only 5 mV. The conclusion must be that the signals shown in the figures cannot be interpreted as IETS signals.

Chemical Reactions in a Frozen Solvent?

In the work of Yang *et al.* [1] the authors claim to have carried out a Michael addition reaction at temperatures below 120 K in ethanol, which freezes at 160 K. Although for rare reactions using water as the medium for synthesis, reactant concentration can increase below 273 K thereby enabling the reaction,[10] to show that the Michael addition reaction proceeds at 160 K in ethanol requires demonstrating this *ex-situ* with standard chemical characterization techniques (NMR, HRMS, HPLC). This was not done by Yang *et al.* [1]

The energetics of this reaction is also problematic. The Michael addition reaction of the 1,3-dicarbonyl compound to maleimide involves breaking a double bond on the maleimide followed by the formation of two single-bonds after a proton transfer from the ethanol solvent. This reaction normally proceeds in the presence of an acid (e.g. trifluoroacetic acid) and a catalyst that ensures a high yield [11, 12]. The reaction barrier for this Michael addition is

16 kcal/mol ($\sim$ 0.7 eV), based on the calculations presented by Yang *et al.* (Figure 2c) [1]. The authors claim further that the theoretical reaction barrier is reduced to 2 kcal/mol (0.09 eV) under an electric field of 2.57 V/nm. Given the junction length ($\sim$ 3.2 nm, set by the length of the molecule), achieving fields as high as 2.5 V/nm would require bias voltages greater than 8 V across the device, assuming that the voltage drops uniformly across the junction. In most molecular junctions, the voltage drop is primarily at the contacts [8, 13] thus even higher biases would be required to achieve fields as high as 2.5 V/nm at the reaction site. The experimental bias of 300 mV only produces the required field of 2.57 V/nm if the gap size were as small as the size of a single hydrogen atom (0.1 nm).

The Michael addition considered in Yang *et al.* has six different possible products, as illustrated in Figure **??**, four of which do not involve a proton transfer from the solvent. In the work of Yang *et al.*, the authors only consider, without justification, two of these six possible products, the ones that include a proton transfer. However, the synthetic procedures [11, 12] clearly state that enantioselectivity and diastereoselectivity can vary widely depending on conditions and substrate identity.

Realtime Monitoring and Chirality Determination: Misinterpretation of Noise

The primary evidence that the authors use to support the claim of observing the Michael addition reaction in real time is the measurement of fluctuations in the current across their device that has the maleimide reactant tethered to it. The authors assume that the current fluctuations result from the 1,3-dicarbonyl compound binding to the maleimide. Such fluctuations in current as a function of time are widely known as random telegraph noise (RTN) and have been studied and observed for many nanoscale systems, and can be attributed to a slew of physical phenomena [14]. Although RTN signals could result from the proposed reaction taking place within the measurement timescales, seeing RTN does not prove that a chemical reaction is in process. It is much more likely that the RTN results from rearrangements of the atomic scale structure of the device (whatever it is), which can be activated simply by increasing the current (or the voltage bias) across a molecular-scale device.

Control experiments, such as those presented in Supplementary Figure 9, are intended to verify that that the RTN signal is absent in the absence of the reactant. However, this is not a valid control. First, it is a different device. Second, the currents are much lower than those in the experiments shown in Figure 2. Since RTN is known to be activated by current (and voltage) over the junction, a valid control should use the same device at the same current. The currents in Supplementary Figure 9, especially for the lower temperatures, are upto a factor of 30 smaller than those shown in Figure 2. For lower currents RTN is likely to disappear. Note also that the current is different for each temperature in Supplementary Figure 9, and is set systematically lower for decreasing temperature. RTN will be more prominent at low temperatures, and one would need to limit the current bias in order to avoid the switching from appearing.

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Figure 1 Caption
Six different possible products that can be formed from a Michael addition reaction of 1,3-dicarbonyl compound to maleimide. The box highlights the two considered in the work of Yang *et al.*, which both require a proton transfer.