Comment on "Following molecular mobility during chemical reactions: no evidence for active propulsion" and "Molecular diffusivity of click reaction components: the diffusion enhancement question"

Tian Huang[1] and Steve Granick[1,2]*

[1]Center for Soft and Living Matter, Institute for Basic Science (IBS), Ulsan 44919, South Korea

[2]Departments of Chemistry and Physics, Ulsan National Institute of Science and Technology (UNIST), Ulsan 44919, South Korea

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ABSTRACT: We provide arguments why we consider as inaccurate two recent JACS Communications which disagree with this laboratory's report of boosted diffusion during the copper-catalyzed azide-alkyne cycloaddition click reaction (CuAAC). Fillbrook et al. claim that their diffusion NMR experiments offer no evidence for boosted diffusion, but their use of Gd^{3+}-chelates to speed up NMR relaxations times is flawed conceptually, the authors interpreting Gd^{3+}-chelates as inert. Actually, the same features that make gadolinium ions useful as contrast agents in magnetic resonance imaging render them unsuitable for diffusion NMR. Nonetheless, by correctly adjusting technical aspects of measurement, we confirm boosted diffusion even in the presence of this MRI contrast agent. The second skeptical Communication, by Rezaei-Ghaleh et al., compares to a reference state that is not meaningful physically.

In this Comment we provide arguments why we consider as inaccurate two recent JACS Communications[1-2] of disagreement with this laboratory's report of boosted diffusion during the copper-catalyzed azide-alkyne cycloaddition (CuAAC) click chemical reaction.[3] Briefly, the first study (Fillbrook et al.[1]) is flawed conceptually in how it approaches diffusion NMR. Data in the second study (Rezaei-Ghaleh et al.[2]) are broadly consistent with ours while the claimed disagreement compares to a physically unrealistic reference state.

This controversy started with this laboratory's report of boosted diffusion during several common chemical reactions (CuAAC reaction, Diels-Alder reaction, and Grubbs catalyzed ring opening metathesis polymerization, ROMP).[3] Regarding the CuAAC reaction, subsequently we considered the mobility of chemical reaction intermediates,[4] scrutinizing how the phenomenon of boosted diffusion can be disentangled in this reaction that has multiple intermediate steps. That study showed that the boosted diffusion phenomenon is most straightforward to see for the azide reactant, which joins the CuAAC reaction at just one step, but also is evident for the alkyne reactant which joins the CuAAC reaction at multiple intermediate steps, a subtlety we had not understood at the time of the original study.[4]

Now we discuss conceptual flaws in Fillbrook et al.[1] The chemical structure of the Gd^{3+}-chelate used by these authors, Gd-DTPA, is drawn in Fig. 1a. In magnetic resonance imaging (MRI) it is used as contrast agent owing to its powerful paramagnetism (7 unpaired electrons), large magnetic moment and electric charge, such that it interacts strongly with protons inside tissues by continual binding-unbinding to the 8 chelated ligands sites and a 9th coordination site on the core Gd^{3+} ion itself.[10-11] Contrary to the claim by Fillbrook et al. that Gd-DTPA is inert, Cu^{2+} has strong affinity to the DTPA ligand of Gd^{3+}.[12-13] When Gd-DTPA is added to the CuAAC reaction, the solution turns faint blue, indicating that Gd^{3+} coordinates with Cu^{2+} to form a bimetallic complex, probably involving Gd-DTPA, Cu^{2+}, and their ligands, as shown by the absorbance spectra plotted in Fig. 1b. From absorbance spectra measured over a range of $CuSO_4$ concentrations with and without Gd-DTPA, assuming the same extinction coefficient of Cu^{2+} and Cu^{2+} in Gd-DTPA, we calculated the ratio of oxidized to native copper ion, $[Cu^{2+}]/[Cu^{+}]$, plotted in Fig. 1c. The active catalyst in the CuAAC reaction is Cu^{+} ion produced by the reduction of Cu^{2+} with ascorbate. This data shows that by stabilizing Cu^{2+}, Gd-DTPA lowers the Cu^{+} concentration below its nominal value. Fig. 2 shows how this biases diffusion NMR.

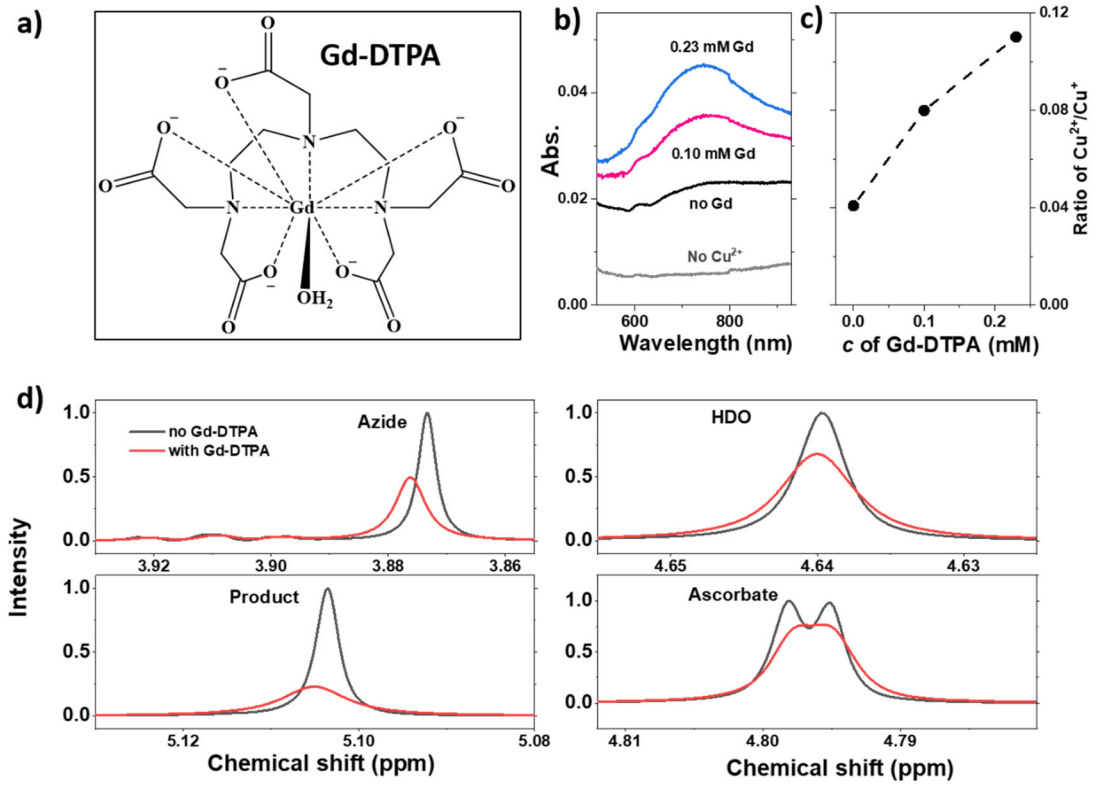

Figure 1. Gd-DTPA and its influence on solution properties of the CuAAC reaction. (a) Chemical structure of diethylenetriaminepentaacetic acid gadolinium (III) dihydrogen salt hydrate, Gd-DTPA. (b) Absorbance plotted against wavelength for the CuAAC reaction mixture used by Fillbrook et al. in ref. 4, except in the absence of $CuSO_4$. (c) The implied ratio, Cu^{2+}/Cu^{+}, plotted against Gd-DTPA concentration. Note that relative to its value in the absence of Gd-DTPA, the presence[1] of 0.23 mM Gd-DTPA more than doubles this ratio. (d) NMR peaks of azide, reaction product, ascorbate, and HDO, in the presence and in the absence of Gd-DTPA. Reaction conditions are the same as in ref. 4.

Other unfortunate implications of adding gadolinium ion offer an alternative explanation of the "artifact" regarding water intensity asserted by Fillbrook et al.[1] An extensive literature from decades ago shows that presence of paramagnetic ions causes NMR peaks to be attenuated, broadened, or not even observable depending on the distance between the nuclei and the paramagnetic ions; this effect is known as a "blind sphere" around the paramagnetic center.[14-15] Both Gd and Cu^{2+} are paramagnetic. The bimetallic paramagnetic complex (Gd-DTPA, Cu^{2+}, and their ligands) can be regarded as a tiny but powerful magnet dispersed in the reaction solution. To this complex, reactant and water molecules bind transiently, sometimes directly to the inner sphere of Gd^{3+}, sometimes to the carboxylate moieties on each DTPA ligand outer sphere.[10] Fig. 1d illustrates that NMR peaks of the azide reactant, ascorbate co-catalyst, HDO solvent, and the reaction product are attenuated and broadened, as the literature leads one to expect; for the alkyne reagent, not shown, it is similar. Binding and unbinding are probably enhanced by the fact that both azide and the reaction product possess three nitrogen atoms with lone pairs, which can facilitate coordination to the Cu-Gd-DTPA complex. Relative to the nine coordination sites already present in Gd-DTPA, the opportunities offered by complexation to the bimetallic complex offer additional sites to which water and other molecules in solution may coordinate, probably explaining why the intensity of the HDO NMR peak is not constant during reaction. Physically, the picture is that water and other molecules continually bind and unbind to Gd-DTPA and the bimetallic complex, their NMR peaks on the experimental time scale being modulated by this process that is so useful for MRI imaging.

Because reagents and water transiently bind and unbind to the bimetallic Cu-Gd-DTPA complex during the CuAAC reaction, the theoretical model used by Fillbrook et al. to analyze their data, the Stejskal-Tanner equation, is oversimplified. Its implicit assumption is that binding-unbinding relaxation processes are negligible on the time scale of the magnetic gradient pulse; in this model, only diffusion causes the diffusing object to deviate from its original spin configuration via diffusion. We hypothesized that binding-unbinding might proceed over the time step that Fillbrook et al. used to perform their diffusion NMR experiments. For this, they employed the magnetic gradient pulse length $\delta = 2.0$ ms, which was a reasonable first guess because it offers a desirable range of attenuated NMR peak intensity to evaluate, but unfortunately this guess was unfortunate. Using the same pulse echo conditions, we have repeated the conditions of their diffusion NMR experiments and confirmed their finding of no evidence for enhanced diffusion of the azide reactant (Fig. 2a), provided one uses their choice of $\delta = 2.0$ ms. But using $\delta = 1.5$ ms shows enhanced diffusion (Fig. 2a), which implies that different δ sample different populations of molecular complexes, each of them presumably heterogeneous. Moreover, by raising the ascorbate concentration we compensated for the fact that the Gd-DTPA tends to deplete Cu^{+} from the mixture (Fig. 2c). In these experiments with catalyst Cu^{+} concentration closer to that typical for the CuAAC reaction, the data show finite enhanced diffusion even for $\delta = 2.0$ ms, and

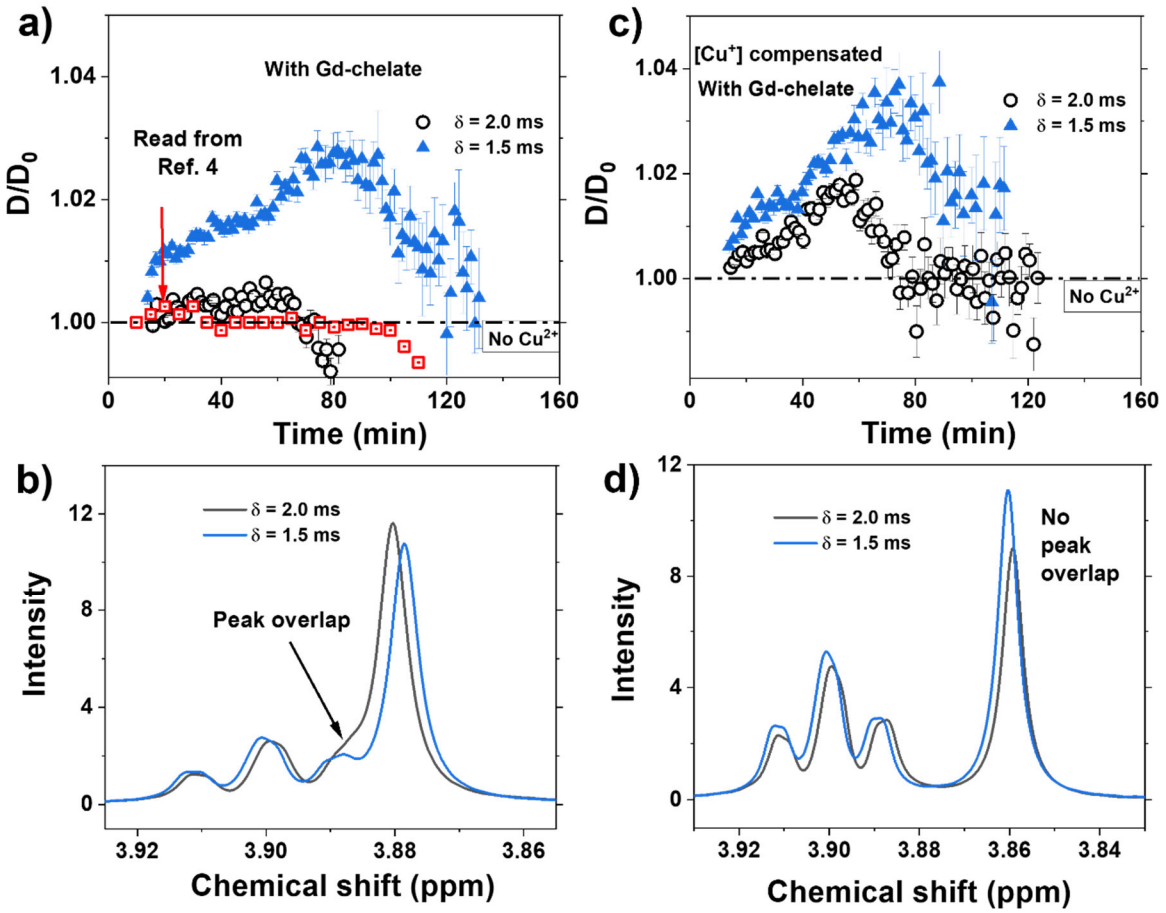

Figure 2. Influence of magnetic gradient duration time. (a) Diffusion coefficient, normalized to its value D_o in the absence of $CuSO_4$, plotted against time, with D_o = 749 $\mu m^2 s^{-1}$. Black: Conditions same as in ref. 4, which employed δ =2.0 ms, except that we adjusted molarity slightly upward to account for our extra filtering step, as specified below. Red: data read from ref. 4. Blue: magnetic gradient duration time δ =1.5 ms. The molar ratio of ascorbate to $CuSO_4$ equals 4. (b) Azide and ascorbate NMR peaks measured at the time of maximum D/D_o in panel a. (c) With ascorbate concentration raised to 150 mM (molar ratio of ascorbate to $CuSO_4$ equal to 6) to compensate for copper complexation by Gd-DTPA, the experiments in panel a were repeated with δ =1.5 ms and δ =2.0 ms. (d) Azide and abscorbate NMR peaks measured at the time of maximum D/D_o in panel b. We now specify the reaction conditions. The starting reaction mixture contains 250 mM alkyne + 300 mM azide + 600 µL D_2O + 100 mM ascorbate + 25 mM $CuSO_4$ + 0.23 mM Gd-DTPA. The solution is filtered by 0.20 µm PTFE filter to remove Cu particles produced by disproportionated reaction. For data in panels c and d, ascorbate concentration is 150 mM. The NMR measurement conditions are as follows. Panels a-d: 600 MHz FT-NMR (Agilent), pulse width = 11.5 µs, relaxation delay time = 3 s, gradient duration = 2.0 ms or 1.5 ms, diffusion time = 50 ms, increasing pulse sequence with convection suppression. Panels e and f: 600 MHz FT-NMR (Agilent), pulse width = 11.0 µs, relaxation delay time = 13 s, gradient duration = 2.0 ms or 1.5 ms, diffusion time = 50 ms, increasing pulse sequence with convection suppression. Integration range of 0.01 ppm was used to obtain the peak intensity.

the difference is accentuated for experiments using δ = 1.5 ms (Fig. 2c). These findings are anticipated by the large literature that, decades ago, used diffusion NMR to study the ligand binding rate[16-19] and probe molecular interactions by affinity NMR[20-21]. Fillbrook et al. preferred instead to explain our measurements as artifact from overlapping peaks of azide and ascorbate in our experiments but not theirs.[4] Their claim is inconsistent with the NMR spectra that we measured in each of these cases (Figs. 2b and 2d, respectively).

To reiterate a point made in the introduction to this paper: the reason that here we highlight data for the azide reactant is simpler analysis; azide joins the CuAAC reaction at just one step, whereas alkyne participates in multiple intermediate reaction steps. Nonetheless, similar arguments hold for the alkyne reagent of the CuAAC reaction, for which the phenomenon of enhanced diffusion can be disentangled using longer analysis as we did elsewhere[4] so this logic is not repeated here.

Turning to the paper by Rezaei-Ghaleh: it assesses boosted diffusion based on comparing to a reference state not meaningful physically. Their reference state is artificial: the reagent in D_2O in the absence of catalyst, co-catalyst, or the second reagent. Instead, the relevant comparison should be the mixture, with and without chemical reaction, because physically this is the more meaningful way to isolate the effects of the chemical reaction.

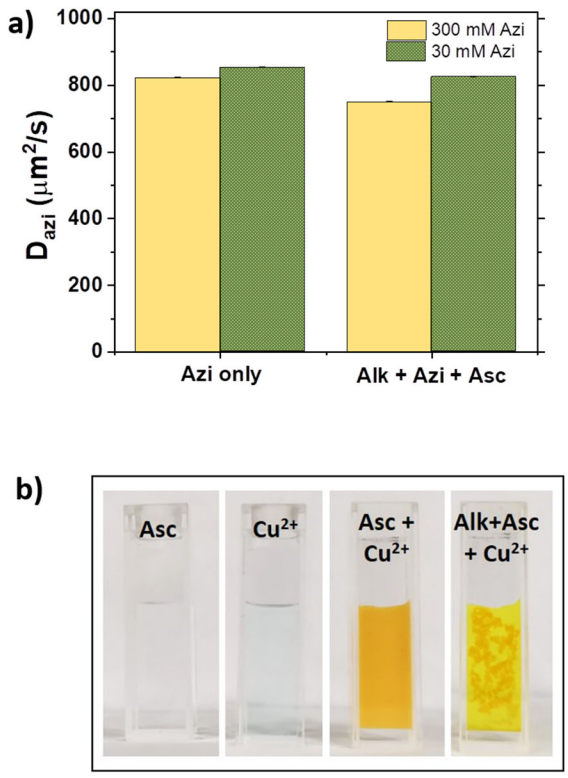

Figure 3. (a) The matter of reference state, D_0, is summarized in a bar graph for the azide reactant. Diffusion coefficient of azide is shown at 300 mM (yellow) and 30 mM (green), contrasting these values for azide alone in D_2O (left), which is the reference state postulated by Rezaei-Ghaleh et al., and for azide in the entire reaction mixture except for absence of $CuSO_4$ absent (right). We hold that D_0 in the mixture, with and without chemical reaction, is more meaningful physically when one seeks to isolate the effects of the chemical reaction. (b)Photographs taken in this laboratory of mixtures regarding which Rezaei-Ghaleh report NMR data: ascorbate (64mM), $CuSO_4$ (16mM), ascorbate + $CuSO_4$ mixture (64 mM + 16 mM), and alkyne + ascorbate +$CuSO_4$ mixture (200 mM + 64 mM + 16 mM), respectively. Upon mixing ascorbate and $CuSO_4$, dark chocolate color was first observed, changing to yellow suspension after around 20 seconds. Upon mixing alkyne, ascorbate and $CuSO_4$, yellow color was observed, then flocculant formed gradually formed. These photos were taken 2 min after vortexer mixing.

Postulating the reference state of an ideal solution free of enthalpic interactions, for which as proxy they use one-component mixtures of each reagent in D_2O, Regaei-Galeh et al.[2] interpret all diffusivity in the CuAAC reaction to signify binding-unbinding equilibria. From this conceptual perspective, faster diffusion of a reagent in the CuAAC reaction signifies that it experiences weaker molecular interactions of some kind (Rezaei-Ghaleh et al. do not explain why chemical reaction alters them), transiently so while the CuAAC reaction proceeds. According to their logic, D(t) should increase monotonically with time, tracking the consumption of reactants by the chemical reaction, but their data do not show this, nor do their data show slowing down of D(t) of the reaction product, which one would anticipate from their logic. It is true that one observes larger base-value diffusion coefficient of the CuAAC reactants when their concentration is lowered, as we illustrate for azide in Fig. 3a, but in a proper comparison the base D_0 should be the mixture environment without chemical reaction, not some hypothetically ideal state without the presence of catalyst, co-catalyst or other reagent.

Interestingly, Rezaei-Ghaleh et al. include NMR data for mixtures that we find to flocculate. The photos in Fig. 3b show alkyne in mixtures with catalyst but no azide present, at the same concentrations used by Rezaei-Ghaleh et al. Flocculation precipitation, obvious in these photos, will affect the concentration and viscosity, making this system unsuitable for diffusion coefficient measurements. Athough their data agree broadly with ours as already noted – our disagreement consisting in the matter of how to normalize the data -- their Fig. 5 cannot be quantitative. When precipitation occurred as time elapsed, reaction concentrations must likewise have changed, but the authors' analysis does not account for this.

These conceptual flaws in Fillbrook et al.[1] and Rezaei-Ghaleh et al.[2] do not support their conclusion that the phenomenon of boosted diffusion is inconsistent with NMR diffusion experiments. In fact, the authors in Fillbrook et al. published two earlier Comments[17-18] to which we replied[19,20], in which they also argued this way. While we understand healthy skepticism because the boosted diffusion phenomenon disagrees with what Philip Ball has called "physicochemical lore",[25] the authors of ref. 1 and 2, having sought to dismiss our arguments, have not provided persuasive reasons. This laboratory finds evidence of boosted diffusion not only for the CuAAC reaction but also other chemical reactions[3] and enzymatic reactions[22-24]. This evidence is supported by independent experimental approaches: not only on diffusion NMR but also microfluidics,[3] fluorescence correlation spectroscopy,[22-24] and dynamic light scattering.[24] We consider this exchange of views to offer additional support that the microscopic consumption of energy by chemical reactions can transduce, under the right circumstances, into mechanical motion manifested as boosted diffusion.[3,4,24]

AUTHOR INFORMATION

Corresponding Author

* Steve Granick - Center for Soft and Living Matter, Institute for Basic Science (IBS), Ulsan 44919, South Korea; Departments of Chemistry and Physics, Ulsan National Institute of Science and Technology (UNIST), Ulsan 44919, South Korea; https://orcid.org/0000-0003-4775-2202; Email: sgranick@gmail.com

Notes

None.

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