Nanophase Structuring in Simple Ternary Solvents Mediates Reaction Kinetics

Yifei Wang[1], Binish Ashfaq[1], Lauren D. Zarzar[1,2,3*]

1. Department of Chemistry, The Pennsylvania State University, University Park, PA 16802, USA
2. Department of Materials Science and Engineering, The Pennsylvania State University, University Park, PA 16802, USA
3. Materials Research Institute, The Pennsylvania State University, University Park, PA 16802, USA

*Corresponding author, ldz4@psu.edu

Key words: nanophases, structured solvent, ternary solvent, click reaction, kinetics, competitive reaction, dynamic light scattering

Abstract

Solvents play a critical role in chemical reactivity. Ternary solvent systems—such as combinations of water, a water-miscible polar organic solvent, and a water-immiscible oil—have been shown to organize into nanophase-structured domains. However, the influence of these nanophases on reaction kinetics remains largely unexplored. In this study, we investigate how nanophase structuring within ternary solvents impacts the kinetics of strain-promoted azide-alkyne click reactions. Ternary solvents were designed to either promote or suppress nanophase structuring, with dynamic light scattering being used to assess nanoscale domain formation. High-throughput UV-Vis kinetic analysis revealed that hydrophobic reactants exhibited significantly enhanced reaction rates within ternary solvents, beyond the hydrophobic effects induced within water-containing binary solvents. Rates were elevated for ternary compositions within a small, specific region of the ternary phase diagram expected to contain oil-in-water nanophases. This kinetic enhancement effect was removed when hydrophilic reactants were used or when nanophase stability was reduced. These findings suggest that the structure of ternary solvent nanophases can be harnessed to modulate reaction kinetics and have influence beyond that of the bulk solvent properties like the polarity or water-induced hydrophobic interactions between reagents. This insight has broad implications for solvent design in synthetic chemistry, biocatalysis, and prebiotic chemistry, offering insight into controlling chemical reactivity in complex, multicomponent solvent systems.

Introduction

Solvents are far more than a medium for dissolving reagents; they can profoundly influence many aspects of chemical reactions, including equilibria, kinetics, and selectivity[1-2]. For example, solvent-solute intermolecular interactions (e.g., hydrogen bonding, counter ion interactions, electron pairing), may influence reagent or catalyst activity, product stability, transition state/intermediates stability, or reaction pathways. Water in particular imparts many nuanced reaction effects[3-6] from introducing hydrophobic interactions[7-9] to hydrogen bonding and interfacial effects (e.g. “on water” catalysis[3, 10]). Using non-reactive self-aggregating molecules like micelle-forming surfactants can also exert influence over reactivity[11-14] by providing nanoscale solvating environments that differ from the bulk. Mixing solvents to create a binary solvent is commonplace for balancing parameters like reagent solubility[15], solvent polarity, or hydrophobic effects[16-17]. Uncovering principles by which simple changes to a solvent, either through chemical composition or molecular organization, can bias chemical reactivity is valuable[18]. Here, we explore the influence of oil / water / cosolvent ternary solvent mixtures formulated to contain nanophase-structured domains on reaction kinetics using alkyne-azide click reactions as probes.

It has been increasingly recognized that macroscopically single-phase ternary solvents, such as those combining water, a water-miscible polar organic solvent, and a water-immiscible oil, can exhibit thermodynamically stable nanophases[19-23]. This solvent “structuring” goes by many names in the literature, including surfactant-free microemulsions[21], ultra-flexible microemulsions[24], pre-Ouzo region[24], mesoscale structuring[25], etc. The solvent nanophases may consist of water-in-oil or oil-in-water pockets, or a bicontinuous structure, with domains on approximately the 1-100 nm scale, dependent upon the formulation and the intermolecular interactions present[20-21]. The main takeaway is that within the single-phase region of a ternary solvent phase diagram, the solvent can assemble into fluctuating nanoscale, heterogeneous domains; do these domains affect chemical reactivity? There have been few studies to address this

https://doi.org/10.26434/chemrxiv-2025-tr75v ORCID: https://orcid.org/0000-0002-3287-3602 Content not peer-reviewed by ChemRxiv.

question. Most notably, such ternary solvents have been explored for biocatalysis, wherein enzymes have been shown to maintain activity in otherwise denaturing solvents due to segregation into water-in-oil nanophases[26-28]. Rates of hydrolysis of nitrophenyl esters[29-30] and chlorophyll[31] were shown to vary as a function of ternary solvent composition with the highest rates achieved in compositions where it is hypothesized that water-in-oil pockets are present. However, in both systems, there are complicating factors such as pH dependence, ionic strength of the solvent, and the fact that water is both a reagent and solvent. Moreover, rates of reactions involving inorganic compounds including interactions of metal ions (e.g., Cu^{2+}) with organic molecules (e.g., *meso*-tetraphenylporphine)[32] or metal complexes hydrolyzing or reacting with cyanide or base[33] were evaluated in ternary solvent systems but with mechanisms focusing either mainly on the reagents interacting at the fluid-fluid interface or effects of phase-transfer catalysis synergistic with reagent concentration fluctuations. Thus, it is still largely unknown how the nanophase organization of solvents influences reaction kinetics for simple reactions that are relatively independent of other physiochemical factors.

In this research, we explore the relationship between ternary solvent composition, nanophase structure, and the kinetics of strain-promoted azide-alkyne click (SPAAC) reactions between cyclooctynes and azides. SPAAC reactions are robust reactions that proceed under mild reaction conditions (e.g., room temperature, neutral pH, no catalyst) and have no side reactions in the presence of water. Ternary solvents were formulated to either promote structuring (1-octanol / isopropanol / water) or disfavor structuring (1-octanol / acetonitrile / water; hexane / isopropanol / water). The ternary solvent nanophase structure was characterized by dynamic light scattering (DLS). High-throughput ultraviolet–visible spectroscopy (UV-Vis) kinetic analysis was performed to characterize the kinetics of the reaction as a function of hundreds of different solvent compositions in the macroscopically single-phase region of each ternary phase diagram. Compared to single and binary solvent compositions, ternary solvent mixtures containing oil-rich nanophase structures showed significant reaction rate elevation when a hydrophobic azide and alkyne reagent pair was used. The nanophase-induced rate enhancement exceeded that from the hydrophobic effects present in the water-containing binary solvent by over five times in some cases. However, if the azide chosen was hydrophilic (e.g. pegylated) or the nanophase stability was dampened (by using weak or non-hydrogen-bonding cosolvent or oil) the kinetic enhancement disappeared. Ternary solvent formulation could be used to bias selectivity in a competitive reaction between hydrophobic and hydrophilic azides reacting with hydrophobic alkyne. Given that this SPAAC reaction was shown to be independent of organic solvent polarity (e.g. rate is identical in the oil and cosolvent) and that the rates in a ternary solvent exceeded that of any water-containing binary solvent, we conclude that the nanophase organization of the ternary solvent is responsible. Interestingly, there is a very specific region of the ternary phase diagram that shows rate elevation, and simply having nanophases is in and of itself insufficient. We discuss how the concentration and composition of the nanophases might play a role. This work sheds light on how simple, ternary solvent mixtures in the absence of any surfactants can be harnessed to influence reaction kinetics. Understanding how to formulate multicomponent solvents with an eye towards controlling their nanoscopic organization in addition to their bulk solvating properties may provide a useful mechanism for intentionally modifying kinetics or selectivity. Further, such investigations can yield fundamental insights into how chemical reactions proceed in complex mixtures[34], which is particularly relevant to understanding the properties of chemical reaction systems / networks[35-36] and how chemical systems may molecularly evolve and give rise to life[37-38].

Results and Discussion

To probe the impact of solvent nanophases on reaction kinetics, we selected a ternary solvent mixture expected to contain structuring: water, 1-octanol, and isopropanol (IPA). The ternary phase diagram, showing multiphase and macroscopically single-phase regions (i.e. homogeneous by eye), is depicted in **Figure 1a**. We used dynamic light scattering (DLS) to probe the nanoscopic solvent structuring across the single-phase region, particularly in compositions near the miscibility gap, where prior research suggests structuring to be prominent[21, 39-40]. DLS autocorrelation curves provide information about the nanophase hydrodynamic diameter and concentration (**Figure 1b**, **Figure S1**). Higher correlation coefficients at the shortest delay times (i.e. the Y intercept) indicate better defined and higher concentrations of scattering nanophases. While small, low concentration, and fast-fluctuating molecular clusters that are beyond the limit of detection by DLS can be characterized by other means such as neutron scattering[41], DLS serves as an accessible and rapid method to screen for solvent structuring[23, 42-44]. Many ternary compositions, particularly those on the water-rich side of the diagram, exhibited strong scattering in DLS. Based on the

DLS data and solvent viscosity, we estimated that the nanophases have hydrodynamic diameters in the range of 1.3 – 4.7 nm (**Table S1**). Pure solvents, as expected, showed no scattering and no structuring (**Figure S1**). Binary solvent mixtures of IPA and water yielded either undetectable or very weak scattering (**Figure 1b**).

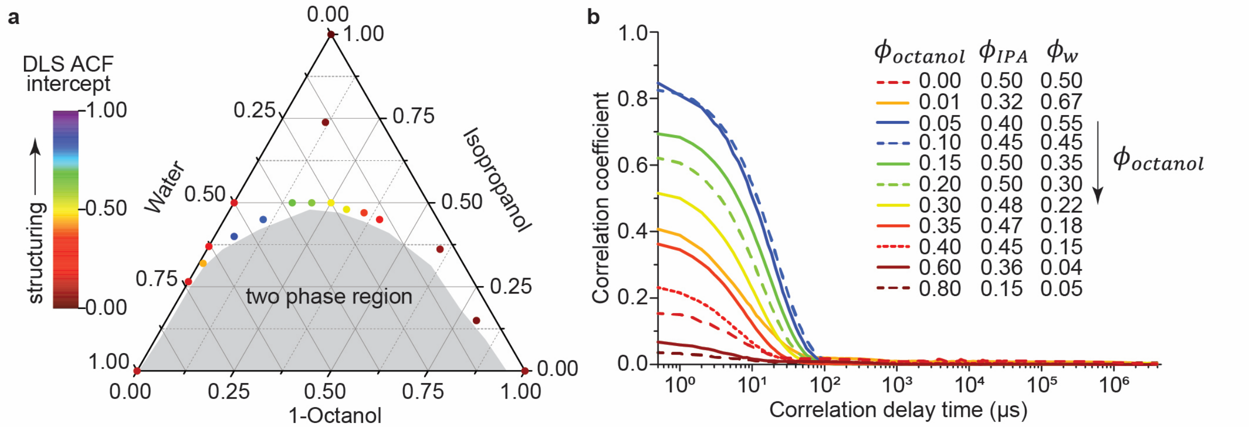


Figure 1. DLS analysis of ternary mixtures of 1-octanol / IPA / water reveals nanophase solvent structuring. (a) Ternary phase diagram for water, 1-octanol, and isopropanol, by volume. Selected compositions were analyzed by DLS to deduce nanophase structuring, and autocorrelation curves are given in **(b)**. The curves in **(b)** and the dots in **(a)** were color coded using the same color scale to indicate the intercept of the autocorrelation function (ACF). For (b), n≥3; PDI standard deviation < 0.02. The estimated hydrodynamic diameter for selected compositions is in **Table S1**.

Having devised a ternary solvent composition, we next needed a reaction to probe kinetics as a function of solvent structure. We wanted to use an un-catalyzed reaction that proceeds at room temperature (RT), is suitable for high-throughput UV-Vis kinetic analysis, has no side reactions, is compatible with aqueous solvent, and exhibits relatively little sensitivity to solvent polarity. We decided to use strain promoted azide-alkyne click (SPAAC) reactions[45-48] between cyclooctynes and azides to yield triazole products. We first explored the reaction between 11,12-didehydro-ε-oxodibenz[b,f]azocine-5(6H)-hexanoic acid (DBCO-C6-acid) (0.11 mg/mL, 0.32 mM, 0.064 μmol) and benzyl azide (0.53 mg/mL, 4.0 mM, 0.80 μmol) at RT within the ternary solvent system 1-octanol, IPA, and water of varying volume fractions (**Figure 2a**). DLS of a 1-octanol / IPA / water mixture (0.10 / 0.45 / 0.45 by volume) with and without reagents indicated that neither these concentrations of reagents nor their product disrupted the nanophase structure (**Figure S2**). We quantified the kinetics of DBCO-C6-acid consumption across many solvent compositions by monitoring the decrease in absorbance at 309 nm using a microplate reader and 96 well plates. The reaction is second order, but we used 12.5x molar excess of benzyl azide to enable the application of pseudo-first order reaction principles and deduced an apparent rate constant, k, for each solvent condition (**Figure S3**). Details are described in the Supporting Information section “Apparent rate constant determination” and the raw data including standard deviation is in **Table S2**. Each solvent condition was analyzed at least three times, and the average k as a function of the solvent composition was plotted onto the phase diagram in **Figure 2b**. The same data from **Figure 2b** was re-plotted in **Figure 2c** as a function of water volume fraction, ϕ_w.

We considered the kinetic data in **Figure 2b,c**. As a control, in pure IPA, k = 0.036 ± 0.0065 mM^{-1} min^{-1} which is the same within error as in pure 1-octanol (0.032 ± 0.015 mM^{-1} min^{-1}), indicating that the reaction is not particularly sensitive to the organic solvent polarity. The reaction in pure water could not be conducted due to solubility limitations. Binary mixtures of IPA and octanol have similar apparent rate constants to the pure octanol and IPA (**Table S2**). The binary mixture of water / IPA exhibits somewhat higher apparent rate constants than pure IPA or octanol ($k \approx$ 0.07 — 0.10 mM^{-1} min^{-1}) which is consistent with hydrophobic effects enhancing reaction rates[45, 49]. More interestingly, the fastest rates by far occur in ternary solvents, wherein some compositions have k > 0.4 mM^{-1} min^{-1}, an order of magnitude higher than in pure IPA or octanol. Some ternary compositions’ k values are 3 to 5 times higher than the corresponding binary solvent with identical ϕ_w (**Figure 2c**). For example, for $\phi_{octanol}$ = 0.003, 0.005, or 0.01 and $\phi_w = 0.7$ to 0.75, k = 0.3 — 0.4 mM^{-1} min^{-1}, whereas water / IPA binary mixtures with identical ϕ_w have k < 0.1 mM^{-1}

min^{-1} (**Figure 2c**). The highest k were found in ternary compositions in the water-rich, oil-poor corner of the phase diagram (yellow and green dots in **Figure 2b**) which correspond to oil-rich nanophase pockets dispersed in a water-rich continuous phase. It appeared that the introduction of an oil, like octanol, within a ternary solvent could be co-localizing the hydrophobic reagents within nanophases and facilitating faster reaction kinetics.

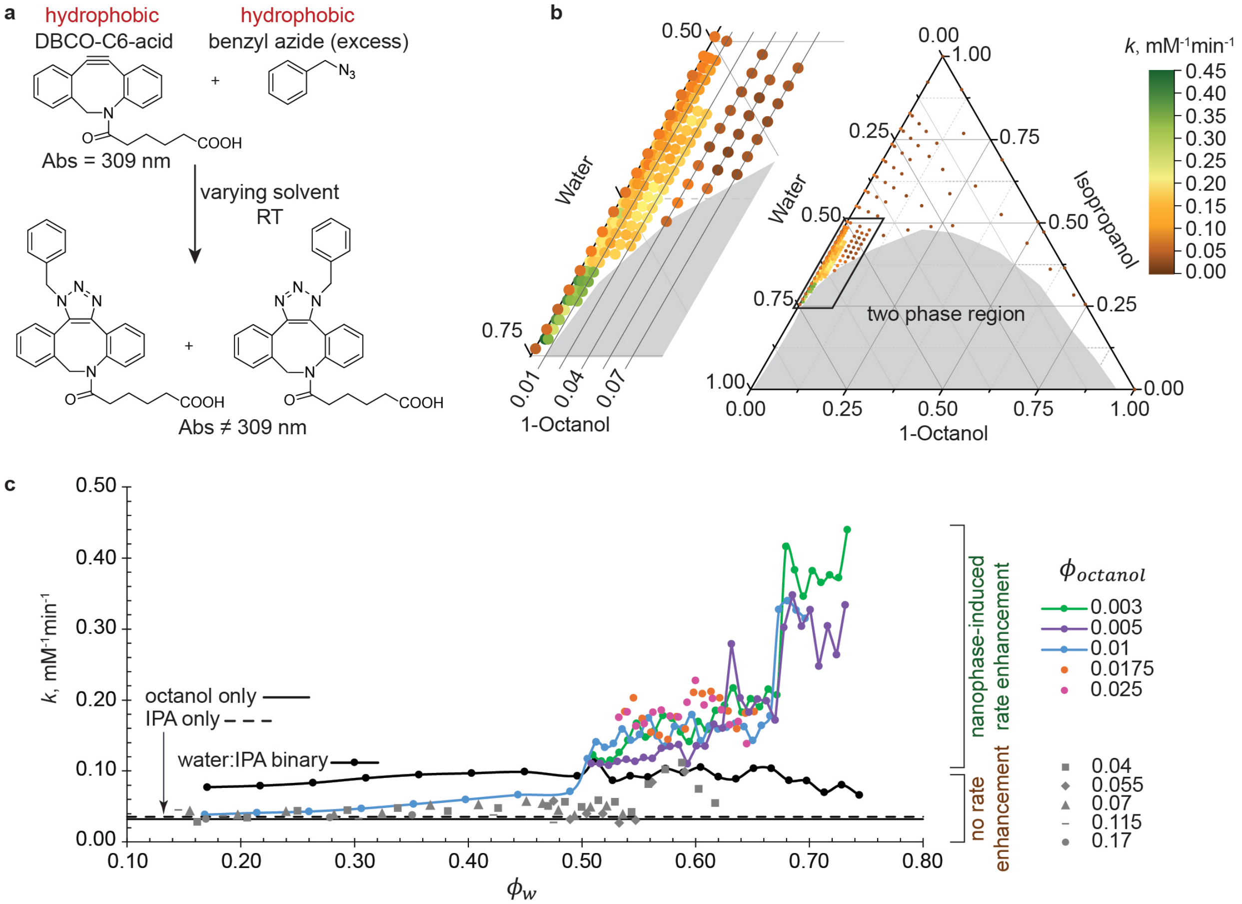


Figure 2. **Kinetic screening of a SPAAC reaction in the 1-octanol / IPA / water solvent reveals elevated apparent rate constants in specific ternary solvent compositions compared to pure or binary solvents. (a)** SPAAC reaction between DBCO-C6-acid and benzyl azide. DBCO-C6-acid absorbs at 309 nm while the products and the benzyl azide do not. Reaction kinetics were determined by monitoring absorbance at 309 nm, reflecting DBCO-C6-acid consumption. **(b)** To induce pseudo-first order kinetics, the reaction in (a) was conducted with 0.11 mg/mL (0.064 µmol) of DBOO-C6-acid and 0.53 mg/mL (0.80 µmol) of benzyl azide at RT under various volumetric mixtures of 1-octanol, IPA, and water. The average apparent rate constant from at least three trials is plotted using a color scale onto the ternary phase diagram. Raw data is in **Table S2. (c)** Data from (b) replotted as a function of ϕ_w, with $\phi_{octanol}$ given in the key. ϕ_{IPA} can be deduced for each data point. k in pure octanol, pure IPA, and the water/IPA binary mixtures are plotted for comparison. Lines guide the eye. The highest k values were observed in ternary solvents.

We wondered if the hypothesized reagent co-localization effects of the nanophases would be dampened if one of the reagents was more hydrophilic. We replaced the hydrophobic benzyl azide with more hydrophilic azide-functionalized polyethylene glycol, m-(PEG)$_4$-azide (0.93 mg/mL, 4.0 mM, 0.80 µmol) (**Figure 3a**). We used the same methods to deduce k as a function of solvent composition for this new reagent scope (**Figure 3b,c, Table S3**). In pure 1-octanol, k = 0.030 ± 0.008 mM^{-1} min^{-1}, and in IPA, k = 0.035 ± 0.007 mM^{-1} min^{-1}. These values are the same as for the reaction of DBCO-C6-acid with benzyl azide, confirming that there is no inherent difference in reactivity for this new reagent pair. However, when using the ternary solvent, we saw overall much lower k when using the hydrophobic-hydrophilic reagent pair (**Figure 3b**) as compared to the hydrophobic-hydrophobic reagent pair (**Figure 2b**). Comparing k for the m-(PEG)$_4$-azide case as a function of ϕ_w, the ternary solvents do not meaningfully accelerate the reaction beyond the hydrophobic-effect-induced acceleration achieved in the water / IPA binary solvent (**Figure 3c**). This observation supports the idea that nanoscale co-localization of reagents into oil-rich nanophases can influence rates, but it requires that both reagents are sufficiently hydrophobic so as to enrich in the oil nanophase pockets, encounter each other, and react.

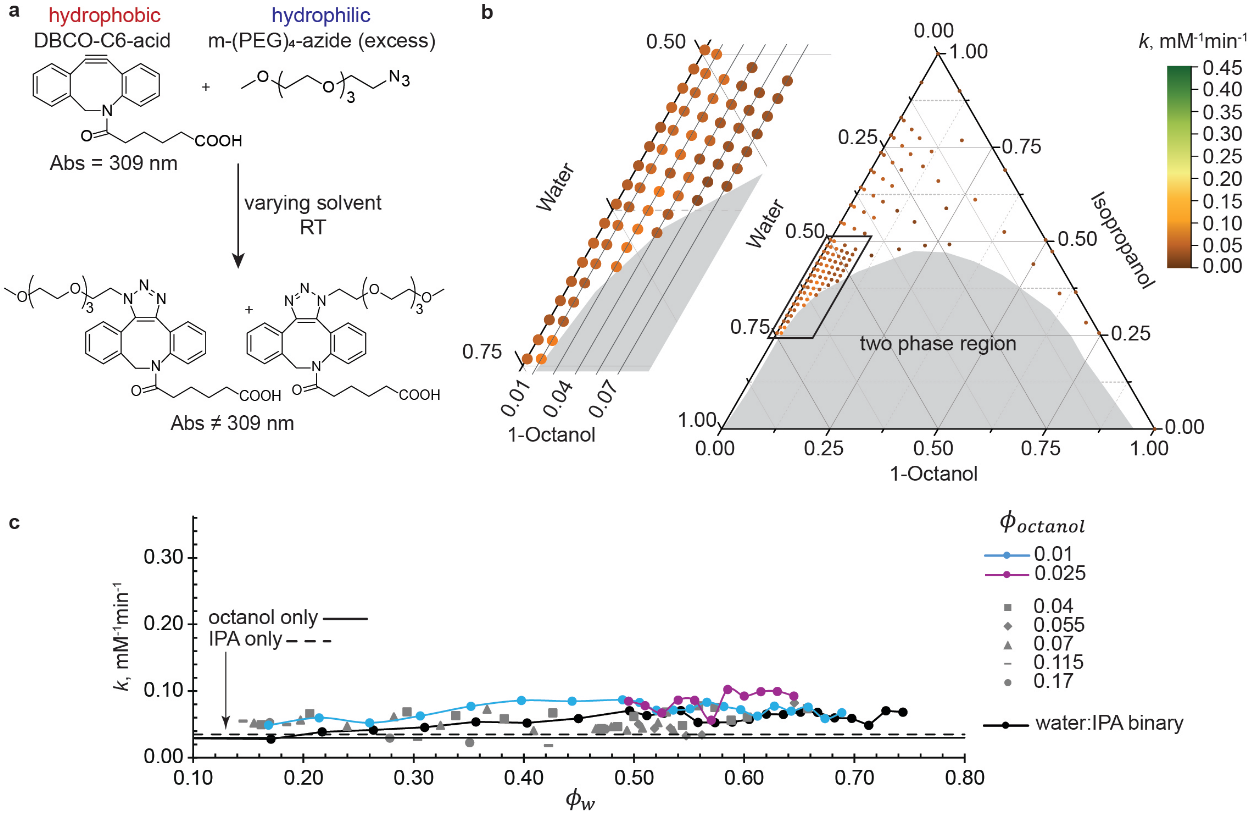


Figure 3. SPAAC reaction between a hydrophobic and hydrophilic reagent is not significantly affected by ternary solvent compositions of 1-octanol / IPA / water. (a) SPAAC reaction between DBCO-C6-acid and m-(PEG)$_4$-azide. DBCO-C6-acid absorbs at 309 nm while the products and the m-(PEG)$_4$-azide do not. Reaction kinetics were determined by monitoring absorbance at 309 nm, reflecting DBCO-C6-acid consumption. **(b)** To induce pseudo-first order kinetics, the reaction in (a) was conducted with 0.11 mg/mL (0.064 µmol) DBOO-C6-acid and 0.93 mg/mL (0.80 µmol) m-(PEG)$_4$-azide. The reaction was conducted at RT under various volumetric mixtures of 1-octanol, IPA, and water and the average k from at least three trials is plotted using a color scale on top of the phase diagram. Raw data is in **Table S3. (c)** Data from (b) replotted as a function of ϕ_w with $\phi_{octanol}$ given in the key. Ternary solvents do not meaningfully accelerate the rate beyond that of the water / IPA binary solvent.

As previously investigated[23, 39, 50-51], the formation and stability of solvent nanophases are influenced by the intermolecular interactions between the oil and cosolvent, such as hydrogen bonding. Ternary mixtures having weaker intermolecular interactions between oil and cosolvent have been shown to have weaker nanophase structuring[23, 39]. We were curious as to how weakening of nanophase structures by reduction in hydrogen bonding, such as by replacing IPA with acetonitrile (ACN)[52-53] or replacing 1-octanol with *n*-hexane, would influence reaction kinetics between two hydrophobic reagents (DBCO-C6-acid and benzyl azide). DLS analysis of 1-octanol / ACN / water solvent, and the *n*-hexane / IPA / water solvent, confirms weaker nanophase structuring (**Figure 4a, Figure S4**) compared to the ternary solvent with 1-octanol / IPA / water (**Figure 1**). For the reaction between DBCO-C6-acid (0.11 mg/mL, 0.32 mM, 0.064 µmol) and benzyl azide (0.53 mg/mL, 4.0 mM, 0.80 µmol) in pure ACN, k = 0.024 ± 0.001 $\text{mM}^{-1}\text{min}^{-}$

^{1} and in pure 1-octanol, k = 0.032 ± 0.015 mM^{-1}min^{-1}, are the same within error, as expected. The reaction in pure *n*-hexane could not be conducted due to solubility limitations. Reactions in the ACN / water binary solvent mixtures have slightly elevated rates, k= 0.033 — 0.12 mM^{-1}min^{-1} that is comparable to IPA / water binary solvent (**Figure 3c**), attributed to the hydrophobic effect. However, as seen in **Figure 4b,c**, and **Figure S5**, there is no significant rate elevation for the ternary compositions compared to the corresponding binary solvent for the 1-octanol / ACN / water or *n*-hexane / IPA / water mixtures. These results also suggest that the presence of more stable nanophases, such as those formed by strong hydrogen bonding between octanol and IPA in the water/ IPA / octanol mixture, influences the nanophase-enhanced kinetic effects.

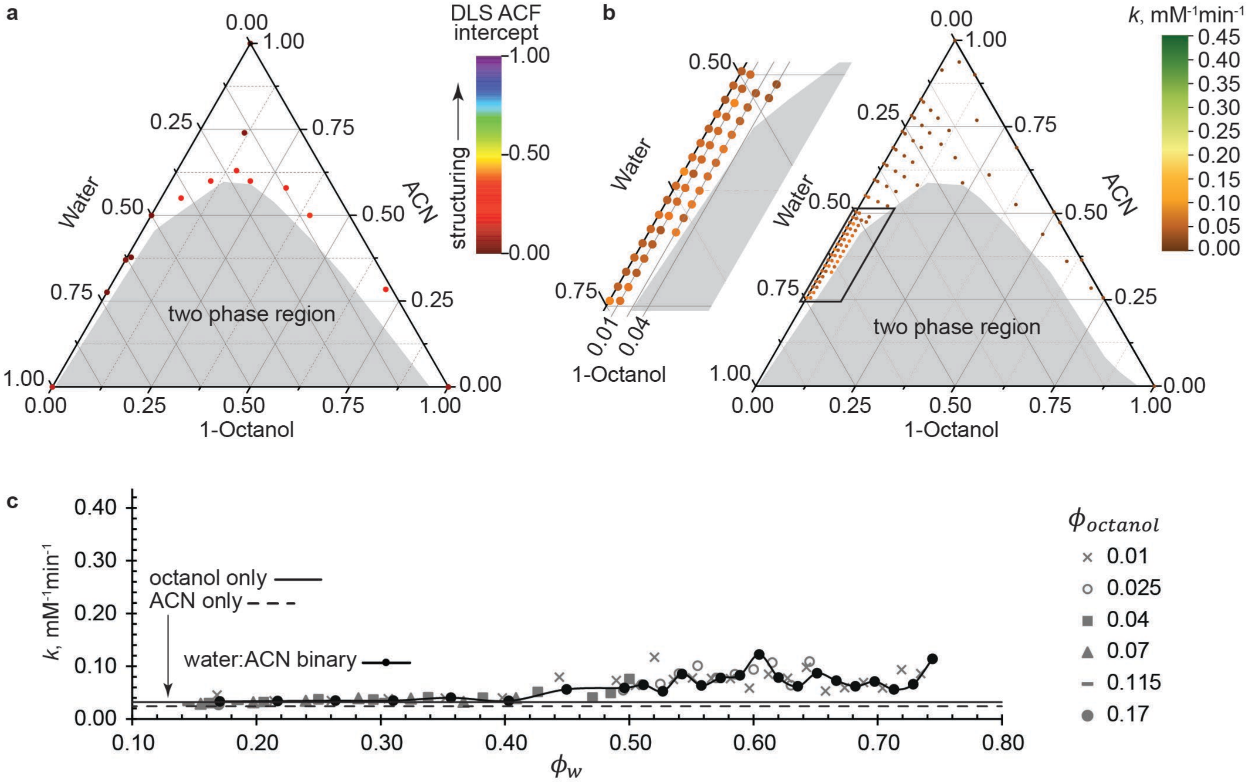


Figure 4. **Weaker nanophase structuring in the 1-octanol / ACN / water solvent shows reduced kinetic enhancement effects for the reaction between DBCO-C6-acid and benzyl azide. (a)** Ternary phase diagram for water, 1-octanol, and ACN, by volume. Colored dots correlate to the intercept of the DLS autocorrelation function (ACF). Exemplary autocorrelation curves are in **Figure S4**. There is less pronounced nanophase structuring compared to 1-octanol / IPA / water. **(b)** To induce pseudo-first order kinetics, the reaction between 0.11 mg/mL (0.064 µmol) DBOO-C6-acid and 0.53 mg/mL (0.80 µmol) benzyl azide was conducted at RT under various volumetric mixtures of 1-octanol, ACN, and water. The average k was deduced from at least three trials at each composition and plotted onto the ternary phase diagram using a color scale. Raw data is in **Table S4. (c)** Data from (b) replotted as a function of ϕ_w with $\phi_{octanol}$ given in the key. ϕ_{ACN} can be deduced for each data point. k in 1-octanol, ACN, and water / ACN binary solvent are plotted for comparison. No rate elevation in the ternary solvent was observed.

It is interesting to consider ways in which simple changes to solvent structure can be used to bias chemical reactivity, particularly under conditions where there is competition for reagents[54]. Given that ternary solvents could be used to increase k for a hydrophobic reagent pair but not a hydrophobic-hydrophilic pair, we thought that the nanophases might provide a route to selectively bias reactivity towards the hydrophobic products. To test this idea, we ran reactions with 0.11 mg/mL (0.32 mM, 0.064 µmol) DBCO-C6-acid, 0.26 mg/mL (2.0 mM, 0.40 µmol) benzyl azide, and 0.46 mg/mL (2.0 mM, 0.40 µmol) m-(PEG)$_4$-azide in various single (IPA), binary (IPA / water) and ternary (1-octanol / IPA / water) solvents. We ran the reaction to completion and used high-performance liquid chromatography (HPLC) to characterize the yield ratio, defined as the mole ratio of benzyl azide-coupled product to m-(PEG)$_4$-azide-coupled product (**Figure**

S6). For each solvent, we also calculated a rate ratio ($k_{benzyl\ azide}/k_{m-(PEG)4-azide}$) using values of k from the non-competitive conditions in **Figure 2, 3, Table S2, S3.** In pure IPA, wherein the rate ratio is 1.02, there was correspondingly no selectivity between benzyl azide and m-(PEG)$_4$-azide (yield ratio = 1.20 ± 0.18). In the ternary solvent ($\phi_{octanol}$ / ϕ_{IPA} / ϕ_w = 0.01 / 0.31 / 0.68), which has a rate ratio of 5.4, the yield ratio was 3.87 ± 0.86, thus favoring the benzyl azide-coupled product as predicted. For the competitive reaction in an IPA / water mixture (ϕ_w = 0.68, ϕ_{IPA} = 0.32) the rate ratio is 1.5 but the yield ratio was notably higher, 3.08 ± 0.45. Thus, it seems likely that there are synergistic hydrophobic effects occurring between the two reactions even in the binary solvents that would be of interest for further exploration.

We considered wholistically the implications of the observed relationships between k, solvents, and reagents in all the systems investigated. The introduction of oil to create a ternary solvent increased reaction rates significantly beyond the water-containing binary solvent when: 1) both reagents were hydrophobic, and 2) the solvent had "stronger" nanophase structuring stabilized by cosolvent-oil hydrogen bonding. Changes in bulk solvent properties like polarity or viscosity, or reagent-solvent intermolecular interactions, do not explain these relationships. Instead, we propose that these data suggest that the presence of well-defined oil-in-water nanophases can co-localize hydrophobic reagents and accelerate reaction kinetics. This nanophase-induced kinetic effect can surpass that of the hydrophobic effects present in the water / cosolvent binary solvent alone. However, even if reagents are hydrophobic and there are nanophases, it is not guaranteed that there is rate elevation. E.g. there are nanophase-containing ternary solvent compositions in **Figure 2** that do not show kinetic enhancement. The fastest rates were found in the bottom left corner of the single-phase region of the phase diagram (low $\phi_{octanol}$ and ϕ_{IPA}, high ϕ_w) so we suspect that the oil-in-water nanophase number density and the "sharpness" of oil-in-water interface may play a role. When the concentration of 1-octanol increases for compositions along the phase boundary, the number density of oil-in-water nanophases is likely increasing; this idea is supported by DLS, wherein the ACF intercept increases with $\phi_{octanol}$ up to ≈ 0.15 (likely indicating more scattering centers, **Figure 1**), yet the hydrodynamic diameter is fairly consistent (**Table S1**). If nanophases become too abundant, the likelihood of reagents encountering each other in the same nanophase will decrease. Analogous kinetic effects have been seen for reactions in surfactant micelles[18, 55-56] (i.e. there is an optimal micelle concentration) and condensates[57-59] (i.e. there is an optimal phase volume ratio). For a given $\phi_{octanol}$, rates were faster when ϕ_w was higher and ϕ_{IPA} was lower; when there is less cosolvent, we expect high partition coefficient of hydrophobic molecules into the oil-rich pockets compared to the water-rich continuous phase. Thus, it appears that there is a localized region within the ternary phase diagram that is optimal for generating the fastest rates.

Conclusions

Here, we employed a high-throughput UV screening methodology to explore how the kinetics of strain promoted azide-alkyne click reactions are influenced by ternary solvent composition and the solvent's nanophase organization. By analyzing the reactivity patterns for hundreds of different solvent compositions, we found that ternary mixtures favoring oil-in-water nanophase structuring, as determined by DLS, can lead to significant increases in reaction rate between two hydrophobic reagents. Rate enhancements exceed that of the binary solvent containing water. In contrast, when a hydrophilic-hydrophobic reagent pair was used, or the ternary solvent was formulated to reduce nanophase stability, the rate enhancement disappeared. We also found that the formation of the ternary solvent can lead to some degree of product selectivity for a competitive reaction between two azides. Our observations suggest that nanophase structuring itself, as opposed to changes in solvent properties like polarity, viscosity, or introduction of hydrogen bonding, can have significant impact on reagent co-localization that facilitates faster kinetics. Notably, the simple presence of oil-in-water nanophases, which were detectable across a broad swath of the phase diagram, was on its own not enough to guarantee faster kinetics; enhanced rates were found within a much smaller range of compositions. We speculate that differences in nanophase concentrations and composition could be playing a role, as similar considerations for balance of effects have been discussed in the context of condensates[57-59] and micellar reactions[18, 55-56]. This research provides insight into how relatively simple, but strategically formulated, solvent mixtures can be used to modulate reaction kinetics via exploitation of nanoscopic heterogeneous environments. These principles may be relevant to systems chemistry[37] broadly, with possible influence on the kinetics and emergent properties of more complex reactive systems like those of autocatalytic networks[60] or oscillatory reactions[61-62], systems involving crystallization[63], and may provide routes to molecular organization that facilitated a matter-to-life transition.

Supporting Information

The supporting Information is available free of charge and contains detailed experimental descriptions of the experimental design and kinetic analysis, supporting figures including DLS, supporting tables containing the raw datapoints presented in the paper, and supporting references.

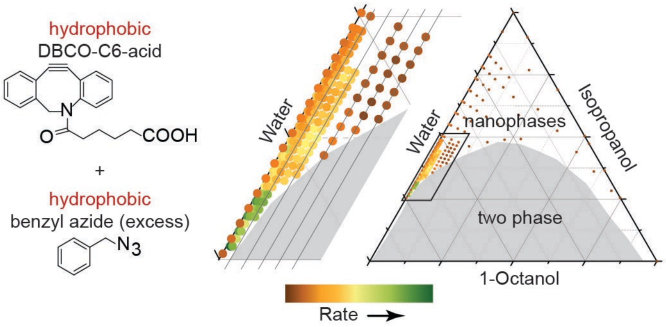
Notes

The authors declare no competing financial interest.

Acknowledgements

All authors acknowledge support from the Alfred P. Sloan Foundation, grant G-2023-19642. All authors thank Dr. Ayusman Sen for helpful discussion. All authors acknowledge the Sheikhi Lab for allowing access to the microplate reader. Y.W. acknowledges Dr. Jian Han, Mr. Rayan Bhattacharya, and Dr. Wangyang Xue for guidance on HPLC.

TOC



References

1. Kononov, L., Chemical reactivity and solution structure: on the way to a paradigm shift? *RSC Advances* **2015,** *5* (58), 46718-46734.
2. Cainelli, G.; Galletti, P.; Giacomini, D., Solvent effects on stereoselectivity: more than just an environment. *Chemical Society Reviews* **2009,** *38* (4), 990-1001.
3. Chanda, A.; Fokin, V. V., Organic synthesis “on water”. *Chemical Reviews* **2009,** *109* (2), 725-748.
4. Li, C.-J.; Chen, L., Organic chemistry in water. *Chemical Society Reviews* **2006,** *35* (1), 68-82.
5. Li, C.-J., Organic reactions in aqueous media with a focus on carbon− carbon bond formations: a decade update. *Chemical Reviews* **2005,** *105* (8), 3095-3166.
6. Cortes-Clerget, M.; Yu, J.; Kincaid, J. R.; Walde, P.; Gallou, F.; Lipshutz, B. H., Water as the reaction medium in organic chemistry: from our worst enemy to our best friend. *Chemical Science* **2021,** *12* (12), 4237-4266.
7. Otto, S.; Engberts, J. B., Hydrophobic interactions and chemical reactivity. *Organic & Biomolecular Chemistry* **2003,** *1* (16), 2809-2820.
8. Lipshutz, B. H.; Ghorai, S.; Cortes-Clerget, M., The hydrophobic effect applied to organic synthesis: recent synthetic chemistry “in water”. *Chemistry–A European Journal* **2018,** *24* (26), 6672-6695.
9. Breslow, R., Hydrophobic effects on simple organic reactions in water. *Accounts of Chemical Research* **1991,** *24* (6), 159-164.
10. Butler, R. N.; Coyne, A. G., Water: nature’s reaction enforcer—comparative effects for organic synthesis “in-water” and “on-water”. *Chemical Reviews* **2010,** *110* (10), 6302-6337.
11. Dwars, T.; Paetzold, E.; Oehme, G., Reactions in micellar systems. *Angewandte Chemie International Edition* **2005,** *44* (44), 7174-7199.
12. Bhat, B. A.; Shairgojray, B. A., Applications of micelles in catalyzing organic reactions. *Mini-Reviews in Organic Chemistry* **2020,** *17* (3), 289-296.
13. Romsted, L. S.; Bunton, C. A.; Yao, J., Micellar catalysis, a useful misnomer. *Current Opinion in Colloid & Interface Science* **1997,** *2* (6), 622-628.

14. Banerjee, M.; Panjikar, P. C.; Bhutia, Z. T.; Bhosle, A. A.; Chatterjee, A., Micellar nanoreactors for organic transformations with a focus on “dehydration” reactions in water: A decade update. *Tetrahedron* **2021,** *88*, 132142.
15. Yu, M. S.; Curran, D. P.; Nagashima, T., Increasing fluorous partition coefficients by solvent tuning. *Organic Letters* **2005,** *7* (17), 3677-3680.
16. Tuulmets, A.; Järv, J.; Tenno, T.; Salmar, S., Significance of hydrophobic interactions in water–organic binary solvents. *Journal of Molecular Liquids* **2009,** *148* (2-3), 94-98.
17. Krickl, S.; Buchecker, T.; Meyer, A. U.; Grillo, I.; Touraud, D.; Bauduin, P.; König, B.; Pfitzner, A.; Kunz, W., A systematic study of the influence of mesoscale structuring on the kinetics of a chemical reaction. *Physical Chemistry Chemical Physics* **2017,** *19* (35), 23773-23780.
18. Serrano-Luginbühl, S.; Ruiz-Mirazo, K.; Ostaszewski, R.; Gallou, F.; Walde, P., Soft and dispersed interface-rich aqueous systems that promote and guide chemical reactions. *Nature Reviews Chemistry* **2018,** *2* (10), 306-327.
19. Blahnik, J.; Müller, E.; Braun, L.; Denk, P.; Kunz, W., Nanoscopic microheterogeneities or pseudo-phase separations in non-conventional liquids. *Current Opinion in Colloid & Interface Science* **2022,** *57*, 101535.
20. Zemb, T.; Kunz, W., Weak aggregation: State of the art, expectations and open questions. *Current Opinion in Colloid & Interface Science* **2016,** *22*, 113-119.
21. Hou, W.; Xu, J., Surfactant-free microemulsions. *Current Opinion in Colloid & Interface Science* **2016,** *25*, 67-74.
22. Chen, C.; Gao, Y.; Li, M.; Sun, C., Unraveling the phase behavior and stability of surfactant-free microemulsions: from molecular interactions to macroscopic properties. *Langmuir* **2024**.
23. Ashfaq, B.; Sen, A.; Zarzar, L. D., Uncovering chemical principles governing nanophase formation in ternary solvents. *Angewandte Chemie International Edition* **2025,** *n/a*, e202421167.
24. Prévost, S.; Krickl, S.; Marčelja, S.; Kunz, W.; Zemb, T.; Grillo, I., Spontaneous ouzo emulsions coexist with pre-ouzo ultraflexible microemulsions. *Langmuir* **2021,** *37* (13), 3817-3827.
25. Sedlák, M. n.; Rak, D., On the origin of mesoscale structures in aqueous solutions of tertiary butyl alcohol: the mystery resolved. *The Journal of Physical Chemistry B* **2014,** *118* (10), 2726-2737.
26. Xenakis, A.; Zoumpanioti, M.; Stamatis, H., Enzymatic reactions in structured surfactant-free microemulsions. *Current Opinion in Colloid & Interface Science* **2016,** *22*, 41-45.
27. Khmelnitsky, Y. L.; Van Hoek, A.; Veeger, C.; Visser, A. J., Detergentless microemulsions as media for enzymatic reactions: spectroscopic and ultracentrifugation studies. *The Journal of Physical Chemistry* **1989,** *93* (2), 872-878.
28. Schöttl, S.; Horinek, D., Aggregation in detergent-free ternary mixtures with microemulsion-like properties. *Current Opinion in Colloid & Interface Science* **2016,** *22*, 8-13.
29. Borys, N. F.; Holt, S. L.; Barden, R. E., Detergentless water/oil microemulsions. III. Effect of KOH on phase diagram and effect of solvent composition on base hydrolysis of esters. *Journal of Colloid and Interface Science* **1979,** *71* (3), 526-532.
30. Bel'skii, V.; Valeeva, F.; Kudryavtseva, L., The influence of detergentless microemulsion on the kinetics of hydrolysis of di (p-nitrophenyl) methylphosphonate catalyzed by amines. *Russian chemical bulletin* **1998,** *47*, 1302-1305.
31. Lavallee, D. K.; Huggins, E.; Lee, S., Kinetics and mechanism of the hydrolysis of chlorophyll a in ternary solvent microemulsion media. *Inorganic Chemistry* **1982,** *21* (4), 1552-1553.
32. Keiser, B.; Holt, S., Reactions in detergentless microemulsions: incorporation of copper (II) into meso-tetraphenylporphine ((TPP) H2) in a water/oil microemulsion. *Inorganic Chemistry* **1982,** *21* (6), 2323-2327.
33. Blandamer, M. J.; Burgess, J.; Clark, B., Enhancement of rates of reaction using neutral water/organic microemulsions as solvent media. *Journal of the Chemical Society, Chemical Communications* **1983,** (12), 659-660.
34. Maguire, O. R., The Many-Chemicals Problem of Systems Chemistry. *ChemSystemsChem* **2024,** *n/a* (n/a), e202400027.
35. Islam, S.; Powner, M. W., Prebiotic systems chemistry: complexity overcoming clutter. *Chem* **2017,** *2* (4), 470-501.
36. Ludlow, R. F.; Otto, S., Systems chemistry. *Chemical Society Reviews* **2008,** *37* (1), 101-108.
37. Ashkenasy, G.; Hermans, T. M.; Otto, S.; Taylor, A. F., Systems chemistry. *Chemical Society Reviews* **2017,** *46* (9), 2543-2554.

38. Mann, S., Systems of creation: the emergence of life from nonliving matter. *Accounts of Chemical Research* **2012,** *45* (12), 2131-2141.
39. Ashfaq, B.; Sen, A.; Zarzar, L., Uncovering chemical principles governing nanophase formation in ternary solvents. **2024.**
40. Klossek, M. L.; Touraud, D.; Zemb, T.; Kunz, W., Structure and solubility in surfactant-free microemulsions. *ChemPhysChem* **2012,** *13* (18), 4116-4119.
41. Buchecker, T.; Krickl, S.; Winkler, R.; Grillo, I.; Bauduin, P.; Touraud, D.; Pfitzner, A.; Kunz, W., The impact of the structuring of hydrotropes in water on the mesoscale solubilisation of a third hydrophobic component. *Physical Chemistry Chemical Physics* **2017,** *19* (3), 1806-1816.
42. Lund, G.; Holt, S., Detergentless water/oil microemulsions: IV. the ternary pseudo-phase diagram for and properties of the system toluene/2-propanol/water. *Journal of the American Oil Chemists' Society* **1980,** *57* (8), 264-267.
43. Fischer, V.; Marcus, J.; Touraud, D.; Diat, O.; Kunz, W., Toward surfactant-free and water-free microemulsions. *Journal of colloid and interface science* **2015,** *453*, 186-193.
44. Xu, J.; Yin, A.; Zhao, J.; Li, D.; Hou, W., Surfactant-free microemulsion composed of oleic acid, n-propanol, and H2O. *The Journal of Physical Chemistry B* **2013,** *117* (1), 450-456.
45. Dommerholt, J.; Rutjes, F. P.; van Delft, F. L., Strain-promoted 1, 3-dipolar cycloaddition of cycloalkynes and organic azides. *Cycloadditions in Bioorthogonal Chemistry* **2016**, 57-76.
46. Mbua, N. E.; Guo, J.; Wolfert, M. A.; Steet, R.; Boons, G. J., Strain-promoted alkyne–azide cycloadditions (SPAAC) reveal new features of glycoconjugate biosynthesis. *ChemBioChem* **2011,** *12* (12), 1912-1921.
47. Dommerholt, J.; Van Rooijen, O.; Borrmann, A.; Guerra, C. F.; Bickelhaupt, F. M.; Van Delft, F. L., Highly accelerated inverse electron-demand cycloaddition of electron-deficient azides with aliphatic cyclooctynes. *Nature Communications* **2014,** *5* (1), 5378.
48. Davis, D. L.; Price, E. K.; Aderibigbe, S. O.; Larkin, M. X.-H.; Barlow, E. D.; Chen, R.; Ford, L. C.; Gray, Z. T.; Gren, S. H.; Jin, Y., Effect of buffer conditions and organic cosolvents on the rate of strain-promoted azide–alkyne cycloaddition. *The Journal of Organic Chemistry* **2016,** *81* (15), 6816-6819.
49. Varga, B. R.; Kállay, M.; Hegyi, K.; Béni, S.; Kele, P., A Non-Fluorinated Monobenzocyclooctyne for Rapid Copper-Free Click Reactions. *Chemistry–A European Journal* **2012,** *18* (3), 822-828.
50. Chen, C.; Gao, Y.; Li, M.; Sun, C., Unraveling the Phase Behavior and Stability of Surfactant-Free Microemulsions: From Molecular Interactions to Macroscopic Properties. *Langmuir* **2024,** *40* (49), 26227-26238.
51. Novikov, A. A.; Semenov, A. P.; Kuchierskaya, A. A.; Kopitsyn, D. S.; Vinokurov, V. A.; Anisimov, M. A., Generic nature of interfacial phenomena in solutions of nonionic hydrotropes. *Langmuir* **2019,** *35* (41), 13480-13487.
52. Marcus, Y., The structure of and interactions in binary acetonitrile+ water mixtures. *Journal of Physical Organic Chemistry* **2012,** *25* (12), 1072-1085.
53. Cringus, D.; Yeremenko, S.; Pshenichnikov, M. S.; Wiersma, D. A., Hydrogen bonding and vibrational energy relaxation in water− acetonitrile mixtures. *The Journal of Physical Chemistry B* **2004,** *108* (29), 10376-10387.
54. Shin, J. A.; Jung, H.; Lim, Y. G., Competitive CuAAC reaction between hydrophobic and hydrophilic alkynes with azides in water. *ChemistrySelect* **2020,** *5* (40), 12371-12376.
55. Menger, F. M.; Portnoy, C. E., Chemistry of reactions proceeding inside molecular aggregates. *Journal of the American Chemical Society* **1967,** *89* (18), 4698-4703.
56. Cordes, E. H.; Dunlap, R. B., Kinetics of organic reactions in micellar systems. *Accounts of Chemical Research* **1969,** *2* (11), 329-337.
57. Smokers, I. B.; Visser, B. S.; Slootbeek, A. D.; Huck, W. T.; Spruijt, E., How droplets can accelerate reactions─ coacervate protocells as catalytic microcompartments. *Accounts of Chemical Research* **2024,** *57* (14), 1885-1895.
58. Smokers, I. B.; Visser, B. S.; Lipiński, W. P.; Nakashima, K. K.; Spruijt, E., Phase-Separated Droplets Can Direct the Kinetics of Chemical Reactions Including Polymerization, Self-Replication and Oscillating Networks. *ChemSystemsChem* **2025,** *7* (1), e202400056.
59. Laha, S.; Bauermann, J.; Jülicher, F.; Michaels, T. C.; Weber, C. A., Chemical reactions regulated by phase-separated condensates. *Physical Review Research* **2024,** *6* (4), 043092.
60. Bissette, A. J.; Fletcher, S. P., Mechanisms of autocatalysis. *Angewandte Chemie International Edition* **2013,** *52* (49), 12800-12826.

61. Semenov, S. N.; Kraft, L. J.; Ainla, A.; Zhao, M.; Baghbanzadeh, M.; Campbell, V. E.; Kang, K.; Fox, J. M.; Whitesides, G. M., Autocatalytic, bistable, oscillatory networks of biologically relevant organic reactions. *Nature* **2016,** *537* (7622), 656-660.
62. ter Harmsel, M.; Maguire, O. R.; Runikhina, S. A.; Wong, A. S. Y.; Huck, W. T. S.; Harutyunyan, S. R., A catalytically active oscillator made from small organic molecules. *Nature* **2023,** *621* (7977), 87-93.
63. Gebauer, D.; Kellermeier, M.; Gale, J. D.; Bergström, L.; Cölfen, H., Pre-nucleation clusters as solute precursors in crystallisation. *Chemical Society Reviews* **2014,** *43* (7), 2348-2371.