First Round of Reviewer Comments

Reviewer: 1

Comments to the Author

In this manuscript, the authors studied the hydrogen binding effect in prototype norbornene (NB) metathesis living polymerization at a single polymer scale. Real-time single-polymer growth studies were realized by applying magnetic tweezers technique. By introducing monomer with -OH group and varying its content ratios, the effects of intra- and inter-chain hydrogen binding on conformational entanglements, aka “hairballs”, were systematically interrogated, accompanying by theoretical modeling. Furthermore, surprising multiphasic dependences with increasing NB-OH percentages regarding the single-polymer growth dynamics were revealed. Theses results have positive significances for both polymer physics and synthetic polymers. The questions and suggestions are listed as follows:

1. Fig. 1b needs to be rearranged for intuitive recognition of messages, i.e. the polymerization equation of 5% NB-OH in a total 1 M NB + NB-OH mixture (gray trace) should be drawn as well; the cartoon schematic better be larger and labeled with “wait” and “jump” process; highlight the grey-box denoting the zoom-in region with red dash line or something for clearly visible. Additionally, the x axis which represents time in seconds is starting with -500, is it any physics significance of minus 500 seconds? Or it should be cut off the minus part of the x axis.

2. In line 42, wrong font size of the word “can”.

3. In line 186, when it comes to “previous discovery”, specific references should be cited for better understanding.

4. It is mentioned, in line 191-197, that the poly(NB-OH) “is supposed to form extensive intra-chain H-bonds”, yet “the conformational entanglements of poly(NB-OH) do not show significant differences in kinetic stability and structural looseness from those of polyNB”. What is the rationale? And also in this part, how to eliminate the effect of intermolecular hydrogen bonds, while exploring the relationship between the intramolecular hydrogen bond and the stability of hairball?

5. Please make sure “versus” or “vs.” was written consistently throughout the main text and figure legends, check line 339, 347, 348, 355, many lines in the paragraph of line 373 to 383, and line 392.

6. The TOC Graphic should be presented in a more exquisite version.
7. Carefully check the typos in the main text, i.e. in line 369 “only from one H-bond” should be “only form”.

8. Check the errors in the references, i.e. line 506 “Macromolecules 2000” should be “Macromolecules 2001”.

9. The authors used NB and NB-OH to study the effect of hydrogen bonding in conformational entanglements on single polymer growth. However, the CH2OH group of NB-OH renders structural perturbation to the NB moiety for polymerization. 2-methyl-5-norbornene (NB-CH3) has almost the same structural perturbation to the NB moiety, so I suggest that NB-CH3 should be added into the discussion to exclude the effect of structural perturbation on polymerization.

10. As the authors mentioned, OH group could potentially bind to the Grubbs catalyst’s Ru center to impede the catalysis. The authors performed the controlled experiment using 0.2 M rNB-OH with 5% NB-OH, the percentage of OH in the polymer chain remains the same as that of 5% NB-OH. However, the total OH group concentration in the solution is 0.25 M, equivalent to that of the 25% NB-OH condition. So, how to exclude OH group effect on catalysis and polymerization kinetics?

Reviewer: 2

Comments to the Author

Baral and co-workers use single-polymer forced-unraveling experiments to examine the transient formation of polymer “hairballs” as a function of hydrogen bonding propensity of the monomer. This is a great example of how a new technique, the authors pulling of living polymers, grants access to exciting new observations, mainly the non-monotonic dependence of polymer dynamics on the mole fraction of the hydroxyl-functionalized monomer. This is a fantastic story. I am particularly excited about the way the authors use their single-molecule perspective to study the dynamics of an “impossible” polymer, one that normally would be insoluble in the medium chosen, but because aggregation is prevented they are able to do experiments. The story is also appropriate for ACS Central science as it will appeal to scientists of many different backgrounds, ranging from materials scientists to chemists to biophysicists (where this pulling technique is more typically applied). I enthusiastically recommend publication after the relatively minor issues described below are addressed.

1. I would like to see more detail about the model (Figure 4 and surrounding text) in the main and SI text. As I understand, the non-monotonicity, which is the central feature of the experimental data, originates in the interplay between the increasing availability of intramolecular hydroxyls due to shifting equilibria, (Fig S13), and the decreasing availability of intramolecular hydroxyls due to the phenomenological function f(x) (Fig S14), which captures the kinetic penalties incurred inside of the hairball. In the main text, the authors should clearly state the origin of the non-monotonicity as originating in these two changing parameters. While I find the use of the f(x) function to be physically intuitive (the fraction of available intramolecular hydroxyls should decrease as x increases since the hairball will be more tightly interconnected), I encourage the authors to find a literature precedent for this phenomenon. I do not think they need a more microscopic model (phenomenological is fine), but rather some supporting literature for the existence of this effect, which is central to the plausibility of
the model. In the SI, the authors should include versions of S15, S16, and S18 where \( f(x) \) is constant (ie, add another line to the figures), to clearly show the centrality of \( f(x) \) to their mechanism.

2. A second issue is the certainty regarding the role of the hairballs in more usual reaction conditions (higher concentration of polymers, no pulling forces). This messaging starts in the abstract, where the hairballs are said to “appear to play key roles in controlling the polymerization kinetics and dispersion.” This type of sentiment appears multiple times in the manuscript, (page 2/line 49; page 3/line 86; page 8/line 247; page 8/line 271). The previous work reported by these PIs in reference 20 is the basis for these claims. While reference 20 is a truly important, landmark experiment, I do not believe it has conclusively shown that hairballs are formed or play “key roles” in typical polymerization conditions. They may play key roles, it has just not been conclusively established. I recommend the authors tone down these claims, and present the hairballs as plausibly, but not unequivocally, playing a key mechanistic role.

3. Figure S16, why are the y-values so different for the blue and red curves? Isn’t this just a matter of having the right a/b/c values? What is the physical significance of the delta concentration being so different between the models?

4. Reference 27 is described as an examination of single metathesis reactions, but that is not correct. The authors may be mixing this paper up with another paper by the same author, which studies small ensembles of metathesis polymerizations in microdroplets, https://doi.org/10.1039/C9SC05559B.

5. Figure 3, the stars and intervals above the figures are not explained. They authors should not rely on the figure 1 caption to explain them.

6. Figure 4b, having the black and blue traces corresponding to the blue left axis is confusing. The authors might make the dashed line blue too.

7. Other minor language issues:

   Line 65, replace “reflects cleanly” with “cleanly reflect”.

   Line 141, something is wrong with this sentence, reword.

   Line 233, but also other places, the authors say “monotonous” or “monotonously” but actually mean “monotonic” or “monotonically”.

   Line 234, replace “a” with “the”

Author’s Response to Peer Review Comments:

Please see the attached point-by-point replies to reviewers attached, and the cover letter.
For reviewer #1:
Thank you very much for your comments and positive evaluation. We have carefully considered your comments and revised the manuscript accordingly. The major changes are highlighted in red in the text. Below are our point-by-point replies to your specific comments.

Recommendation: Publish in ACS Central Science after minor revisions noted.

Comments:
In this manuscript, the authors studied the hydrogen binding effect in prototype norbornene (NB) metathesis living polymerization at a single polymer scale. Real-time single-polymer growth studies were realized by applying magnetic tweezers technique. By introducing monomer with -OH group and varying its content ratios, the effects of intra- and inter-chain hydrogen binding on conformational entanglements, aka “hairballs”, were systematically interrogated, accompanying by theoretical modeling. Furthermore, surprising multiphasic dependences with increasing NB-OH percentages regarding the single-polymer growth dynamics were revealed. Theses results have positive significances for both polymer physics and synthetic polymers.

The questions and suggestions are listed as follows:
1. Fig. 1b needs to be rearranged for intuitive recognition of messages, i.e. the polymerization equation of 5% NB-OH in a total 1 M NB + NB-OH mixture (gray trace) should be drawn as well; the cartoon schematic better be larger and labeled with “wait” and “jump” process; highlight the grey-box denoting the zoom-in region with red dash line or something for clearly visible. Additionally, the x axis which represents time in seconds is starting with -500, is it any physics significance of minus 500 seconds? Or it should be cut off the minus part of the x axis.

[Reply 1] We have revised the Figure 1b to:
- include the general ROMP reaction scheme and label the extension growth trajectories with the respective monomer compositions
- further annotated the inset cartoon with “wait” and “jump”

The negative time scale represents the incubation period during which the sample was placed under high force (~17 pN) for ~10-15 minutes before the monomer addition to ensure the removal of any nonspecifically adsorbed particles and stretch out potential nonspecific interactions between the magnetic particles and the coverslip surface. We have revised the Figure 1b to use a black arrow to mark the monomer addition (i.e., time = 0) and revised the caption to specify this information.

2. In line 42, wrong font size of the word “can”.
[Reply 2] Thank you very much for identifying this error. We have revised the manuscript accordingly and further proofread the writing.

3. In line 186, when it comes to “previous discovery”, specific references should be cited for better understanding.
[Reply 3] Ref. 19 (Science 2017) and 20 (Chem 2021) have been added to support the statement.

4. It is mentioned, in line 191-197, that the poly(NB-OH) “is supposed to form extensive intra-chain H-bonds”, yet “the conformational entanglements of poly(NB-OH) do not show significant differences in kinetic stability and structural looseness from those of polyNB”. What is the rationale? And also in this part, how to eliminate the effect of intermolecular hydrogen bonds, while exploring the relationship between the intramolecular hydrogen bond and the stability of hairball?

[Reply 4] The statement here (page 5) only considered the simplistic prediction that the extensive intra-chain H-bonds in poly(NB-OH) are expected to affect (increase) the kinetic stability of hairballs compared with that of polyNB. The contributions of inter-molecular H-bonds were later considered in the manuscript and further investigated with the introduction of 2-norbornanemethanol (the reduced form of NB-OH). We have added a note in the text (page 5, last paragraph) to refer to later text.

5. Please make sure “versus” or “vs.” was written consistently throughout the main text and figure legends, check line 339, 347, 348, 355, many lines in the paragraph of line 373 to 383, and line 392.
[Reply 5] Thank you very much for identifying this inconsistency. We have revised the manuscript accordingly and further proofread the writing.

6. The TOC Graphic should be presented in a more exquisite version.
[Reply 6] We have tried to improve the TOC graphic.

7. Carefully check the typos in the main text, i.e. in line 369 “only from one H-bond” should be “only form”.
[Reply 7] Thank you very much for identifying this error. We have revised the manuscript accordingly and further proofread the writing.

8. Check the errors in the references, i.e. line 506 “Macromolecules 2000” should be “Macromolecules 2001”.
[Reply 8] Thank you very much for identifying this error. We have revised the reference accordingly.

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[Reply 9] Thank you for the suggestion. Unfortunately, 2-methyl-5-norbornene (NB-CH3) is not commercially available. To introduce H-bonding, we rationalized that the -CH2OH group is small compared with the size of the large norbornene moiety in NB-OH (page 4, paragraph 1). Some structural perturbation might be there, but hard to quantify. More importantly, no multiphasic dependences would be expected from mere structural perturbation. On the other hand, H-bonding is a much stronger interaction in aprotic solvent (page 4, paragraph 1, and SI page S14, approximation 1), and the observed multiphasic dependences on NB-OH% are fully interpretable using intra-/inter-molecular H-bonding interactions. Therefore, we believe H-bonding is the dominant player, while structural perturbations as well as associated possible changes in other van der Waals interactions are less important (SI page S14, approximation 1). We have added a short related discussion on this on page 11, paragraph 4.

10. As the authors mentioned, OH group could potentially bind to the Grubbs catalyst’s Ru center to impede the catalysis. The authors performed the controlled experiment using 0.2 M rNB-OH with 5% NB-OH, the percentage of OH in the polymer chain remains the same as that of 5% NB-OH. However, the total OH group concentration in the solution is 0.25 M, equivalent to that of the 25% NB-OH condition. So, how to exclude OH group effect on catalysis and polymerization kinetics?
[Reply 10] If OH coordination to Ru is important, adding rNB-OH would further inhibit the catalyst and slow down the polymerization, whereas the opposite was observed experimentally, thus ruling out this possibility. The SI Section 4 provides a more detailed discussion on possibilities of OH coordination to Ru, and is cross-cited in the main text (page 11, end of paragraph 1).

Questions:
Quality of experimental data, technical rigor: High
Significance to chemistry researchers in this and related fields: Top 5%
Broad interest to other researchers: High
Novelty: High
Is this research study suitable for media coverage or a First Reactions (a News & Views piece in the journal)?: Yes
For reviewer #2:

Thank you very much for your comments and positive evaluation. We have carefully considered your comments and revised the manuscript accordingly. The major changes are highlighted in red in the text. Below are our point-by-point replies to your specific comments.

Recommendation: Publish in ACS Central Science after minor revisions noted.

Comments:

Baral and co-workers use single-polymer forced-unraveling experiments to examine the transient formation of polymer “hairballs” as a function of hydrogen bonding propensity of the monomer. This is a great example of how a new technique, the authors pulling of living polymers, grants access to exciting new observations, mainly the non-monotonic dependence of polymer dynamics on the mole fraction of the hydroxyl-functionalized monomer. This is a fantastic story. I am particularly excited about the way the authors use their single-molecule perspective to study the dynamics of an “impossible” polymer, one that normally would be insoluble in the medium chosen, but because aggregation is prevented they are able to do experiments. The story is also appropriate for ACS Central Science as it will appeal to scientist of many different backgrounds, ranging from materials scientists to chemists to biophysicists (where this pulling technique is more typically applied). I enthusiastically recommend publication after the relatively minor issues described below are addressed.

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While I find the use of the f(x) function to be physically intuitive (the fraction of available intramolecular hydroxyls should decrease as x increases since the hairball will be more tightly interconnected), I encourage the authors to find a literature precedent for this phenomenon. I do not think they need a more microscopic model (phenomenological is fine), but rather some supporting literature for the existence of this effect, which is central to the plausibility of the model.

In the SI, the authors should include versions of S15, S16, and S18 where f(x) is constant (ie, add another line to the figures), to clearly show the centrality of f(x) to their mechanism.

[Reply 1] We have now revised the text associated with Figure 4 to include a bit more discussion from the SI text and clearly stated the dominant factors that contribute to the multiphasic behaviors (page 11, paragraph 2 and 3).

To support the use of f(x): (1) In single-chain polymer nanoparticle, which is formed by intra-chain cross linking, it has been shown that the rigidity of the single-chain nanoparticle becomes higher with increasing amount of cross linking (Cherian et al. JACS, 2007, 129, 11350). Therefore, more extensive intra-chain interactions make the chain more rigid, which supports that with more OH groups, the fraction of available free OH would be less. (2) Our extension-force measurements show that the persistence length of poly(NB-OH) is higher than polyNB, supporting that the structural rigidity is increased by more hydrogen bonding. We have added these discussions in the SI (page S20, paragraph 2).

We have now added a curve with a constant f(x) in Figure S16. Using a constant f(x) cannot converge when fitting the experimental results, demonstrating the necessity of f(x), and consequently, we did not add corresponding curves to Figures S15 and S17 (please note there was no Figure S18).

2. A second issue is the certainty regarding the role of the hairballs in more usual reaction conditions (higher concentration of polymers, no pulling forces). This messaging starts in the abstract, where the hairballs are said to “appear to play key roles in controlling the polymerization kinetics and dispersion.” This type of sentiment appears multiple times in the manuscript, (page 2/line 49; page 3/line 86; page 8/line 247; page 8/line 271). The previous...
work reported by these PIs in reference 20 is the basis for these claims. While reference 20 is a truly important, landmark experiment, I do not believe it has conclusively shown that hairballs are formed or play “key roles” in typical polymerization conditions. They may play key roles, it has just not been conclusively established. I recommend the authors tone down these claims, and present the hairballs as plausibly, but not unequivocally, playing a key mechanistic role.

**[Reply 2]** Besides our earlier work (Science 2017, 358, 352), our more recent work on conjugated polymers further supported the important roles of these entanglements in controlling polymerization kinetics of long-chain polymers (Chem 2021, 7, 2175). But indeed, our experimental conditions are different from typical solution polymerization conditions, including a pulling force, both ends being tethered, the extremely dilute conditions where inter-chain interactions are negligible, minimal monomer depletion, polymerization via ROMP, and the polymerizations we studied all formed long chains. We have revised the wording in the text to denote the context of our observations and the condition differences (page 2, end of paragraph 2). We have also added “plausibly” at a number of places.

3. Figure S16, why are the y-values so different for the blue and red curves? Isn’t this just a matter of having the right a/b/c values? What is the physical significance of the delta concentration being so different between the models?

**[Reply 3]** The y-values are mainly affected by the value of the parameter \( d \) in Eq. 41, which is simply a combined scaling factor. The shapes of the fitted curves are determined by the delta concentration.

4. Reference 27 is described as an examination of single metathesis reactions, but that is not correct. The authors may be mixing this paper up with another paper by the same author, which studies small ensembles of metathesis polymerizations in microdroplets, https://doi.org/10.1039/C9SC05559B.

**[Reply 4]** Thanks for suggesting this Chem. Sci paper, which is now added as Ref 29 as another example of emerging efforts toward studying polymerization reactions of synthetic polymers toward the single-molecule level. Ref 27 (Ref 30 in the revised manuscript) was specified as the study of initiation kinetics of single organometallic catalysts for metathesis reactions.

5. Figure 3, the stars and intervals above the figures are not explained. They authors should not rely on the figure 1 caption to explain them.

**[Reply 5]** We have now added the description to Figure 3 caption as well.

6. Figure 4b, having the black and blue traces corresponding to the blue left axis is confusing. The authors might make the dashed line blue too.

**[Reply 6]** We have revised the Figure 4 per your suggestion.

7. Other minor language issues:
   Line 65, replace “reflects cleanly” with “cleanly reflect”.
   Line 141, something is wrong with this sentence, reword.
   Line 233, but also other places, the authors say “monotonous” or “monotonously” but actually mean “monotonic” or “monotonically”.
   Line 234, replace “a” with “the”

**[Reply 7]** Thank you very much for identifying these errors. We have revised the manuscript accordingly and further proofread the writing.

Additional Questions:
Quality of experimental data, technical rigor: Top 5%
Significance to chemistry researchers in this and related fields: Top 5%
Broad interest to other researchers: Top 1%
Novelty: Top 1%
Is this research study suitable for media coverage or a First Reactions (a News & Views piece in the journal)?: Yes