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pH-Switchable Strand Orientation in Peptide Assemblies

Nathan A. Schnarr and Alan J. Kennan*

Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523

kennan@lamar.colostate.edu

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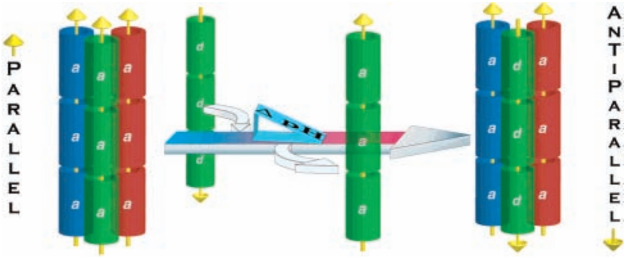
ABSTRACT



The design of antiparallel coiled-coil heterotrimers with singly mismatched electrostatic interfaces is reported. The new complexes exhibit expected properties for well-formed coiled-coils and have stabilities comparable to those of parallel analogues. The mismatched interface facilitates switching from a parallel to antiparallel complex by pH-triggered strand exchange.

Protein conformational changes, ligand exchanges, and other structural adjustments, particularly in response to external stimuli, are central to numerous cellular processes. Replication of such dynamic behaviors in simplified peptide assemblies can illuminate their fundamental controlling factors. Such systems can also provide building blocks for the design of stimulus responsive biomaterials. Here we describe dynamic switching of a parallel coiled-coil trimer to an antiparallel one, triggered by pH-induced strand exchange.

The α-helical coiled-coil, composed of two or more individual polypeptide chains assembled with a superhelical twist, is a particularly attractive arena for testing principles of peptide assembly. Its broad application in biology has inspired many synthetic mimics, and much is now known about its basic structure.[1] The highly regular primary sequence exhibits a heptad repeat (*abcdefg*), and interhelical assembly is governed largely by hydrophobic core *a*/*d* side chains and hydrophilic *e*/*g* ones. Parallel complexes contain alternating *a* and *d* core layers, with electrostatic interfaces between *e* and *g* positions. Antiparallel structures, in contrast, exhibit mixed *a*/*d* core layers and different electrostatic contacts (one *e*/*e*, one *g*/*g*, and one *e*/*g* in the case of trimers). Designed antiparallel coiled-coils are less common than parallel ones, in part due to difficulties with natural sequences as starting points.[2]

We have previously described the use of core residue steric matching to control stoichiometry and strand orientation in trimeric coiled-coils. Preferred 2:1 packing of alanine/cyclohexylalanine side chains at three consecutive *a* positions affords specific, parallel, 1:1:1 heterotrimers.[3] At least two of the three *e*/*g* interfaces must be electrostatically matched

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(1) Reviews: (a) Micklatcher, C.; Chmielewski, J. *Curr. Opin. Chem. Biol.* **1999**, *3*, 724−729. (b) DeGrado, W. F.; Summa, C. M.; Pavone, V.; Nastri, F.; Lombardi, A. *Annu. Rev. Biochem.* **1999**, *68*, 779−819 and references therein.

(2) (a) Review: Oakley, M. G.; Hollenbeck, J. J. *Curr. Opin. Struct. Biol.* **2001**, *11*, 450−457. (b) Gurnon, D. G.; Whitaker, J. A.; Oakley, M. G. *J. Am. Chem. Soc.* **2003**, *125*, 7518−7519. (c) Kim, B.-M.; Oakley, M. G. *J. Am. Chem. Soc.* **2002**, *124*, 8237−8244. (d) McClain, D. L.; Woods, H. L.; Oakley, M. G. *J. Am. Chem. Soc.* **2001**, *123*, 3151−3152. (e) Ghosh, I.; Hamilton, A. D.; Regan, L. *J. Am. Chem. Soc.* **2000**, *122*, 5658−5659. (f) Oakley, M. G.; Kim, P. S. *Biochemistry* **1998**, *37*, 12603−12610. (g) Lombardi, A.; Bryson, J. W.; DeGrado, W. F. *Biopolymers* **1996**, *40*, 495−504. (h) Betz, S. F.; DeGrado W. F. *Biochemistry* **1996**, *35*, 6955−6962. (i) Monera, O. D.; Zhou, N. E.; Lavigne, P.; Kay, C. M.; Hodges, R. S. *J. Biol. Chem.* **1996**, *271*, 3995−4001. (j) Lumb, K. J.; Kim, P. S. *Biochemistry* **1995**, *34*, 8642−8648. (k) Monera, O. D.; Kay, C. M.; Hodges, R. S. *Biochemistry* **1994**, *33*, 3862−3871. (l) Lovejoy, B.; Choe, S.; Casico, D.; McRorie, D. K.; DeGrado, W. F.; Eisenberg, D. *Science* **1993**, *259*, 1288−1293. (m) Monera, O. D.; Zhou, N. E.; Kay, C. M.; Hodges, R. S. *J. Biol. Chem.* **1993**, *268*, 19218−19227.

(3) (a) Schnarr, N. A.; Kennan, A. J. *J. Am. Chem. Soc.* **2002**, *124*, 9779−9783. (b) Schnarr, N. A.; Kennan, A. J. *J. Am. Chem. Soc.* **2001**, *123*, 11081−11082.

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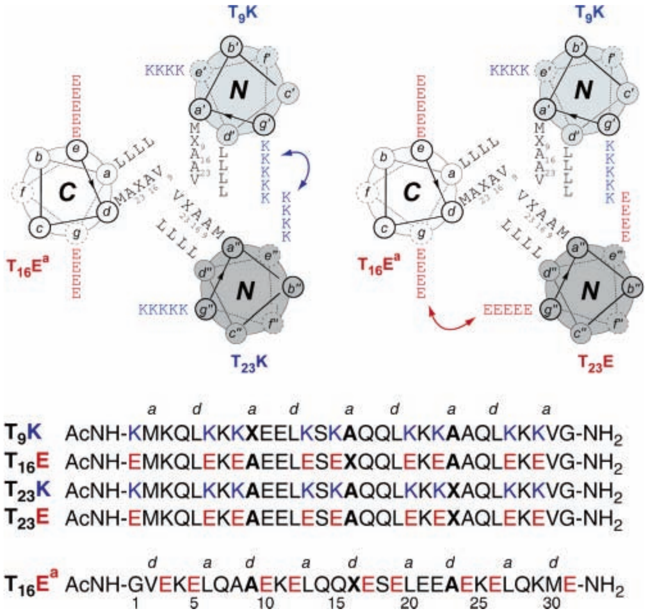

Figure 1. Peptide sequences and trimer helical wheel projections. Peptides are named after parent T$_9$, T$_{16}$, T$_{23}$ sequences, where *n* refers to cyclohexylalanine position. The E or K label designates the amino acid identity at all *e*/*g* positions (Glu or Lys, respectively). In T$_{16}$E^a, core substitutions occur at *d* (rather than *a*) heptad positions. Wheel projection looks down on N-termini of T$_9$K, T$_{23}$E or T$_{23}$K, and C-terminus of T$_{16}$E^a.

(Glu paired with Lys), while the third can be either Glu/Lys or Lys/Lys at neutral pH, or Glu/Glu at low pH.[4]

Recently, we have reported that mixing of two *a* substituted peptides with one bearing *d* core substitutions results in an antiparallel coiled-coil trimer, with fully matched electrostatic contacts.[5] Given our previous experience with pH manipulation of parallel single *e*/*g* mismatch complexes, we sought to investigate the feasibility of analogous antiparallel structures, and in particular whether switching from a parallel to antiparallel trimer was possible, using pH-triggered strand exchange.

The peptides employed have been previously reported (Figure 1).[4,5] Each contains two alanines and one cyclohexylalanine at core positions 9, 16, and 23, in one of three possible arrangements (AAX, AXA, XAA, with X = cyclohexylalanine). The *e*/*g* positions contain either all glutamic acids (E peptides) or all lysines (K peptides). All of the heterotrimers discussed below combine T$_9$K with either T$_{23}$E (Glu/Glu mismatched complexes) or T$_{23}$K (Lys/Lys mismatched). The choice of parallel versus antiparallel arrangement is made by selection of the T$_{16}$ derivative; incorporation of T$_{16}$E affords parallel complexes, while use of T$_{16}$E^a (core substitution in *d* rather than *a* positions) results in antiparallel structures.

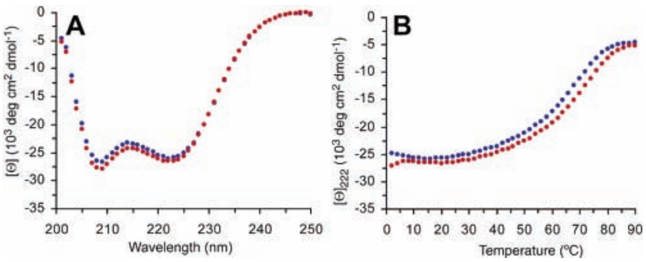

Figure 2. CD data for single Lys/Lys mismatch complexes. Wavelength (A) and thermal denaturation (B) data for solutions of T$_9$K:T$_{16}$E:T$_{23}$K (parallel, red circles) and T$_9$K:T$_{16}$E^a:T$_{23}$K (antiparallel, blue circles). All solutions are 10 μM total peptide in PBS buffer (10 mM phosphate, pH 7.5, 150 mM NaCl). Wavelength spectra taken at 25 °C.

Initial viability of the single-mismatch antiparallel trimers was probed by circular dichroism (CD) spectroscopy. A 1:1:1 T$_9$K:T$_{16}$E^a:T$_{23}$K mixture (Lys/Lys mismatch) exhibits the expected minima at 208 and 222 nm, and its helicity compares favorably with that of a T$_9$K:T$_{16}$E:T$_{23}$K solution, which should form the related parallel complex ($[\theta]_{222} = -26\,030$ versus $-26\,550$, Figure 2a).[6] Both complexes also display similar cooperative unfolding curves and melting temperatures in thermal denaturation experiments ($T_m = 73$ versus 75 °C, Figure 2b). Investigation of equimolar T$_9$K:T$_{16}$E^a:T$_{23}$E and T$_9$K:T$_{16}$E:T$_{23}$E solutions demonstrates that an antiparallel single Glu/Glu mismatch trimer and its parallel analogue are also comparable ($[\theta]_{222} = -30\,421$ versus $-31\,646$, $T_m = 81$ versus 87 °C, Figure 3). Both antiparallel complexes are trimeric by analytical ultracentrifugation ($M_{r,obsd} = 11\,283$, $M_{r,calcd} = 11\,559$ for 1:1:1 T$_9$K:T$_{16}$E^a:T$_{23}$K; $M_{r,obsd} = 11\,764$, $M_{r,calcd} = 11\,568$ for 1:1:1 T$_9$K:T$_{16}$E^a:T$_{23}$E).[7]

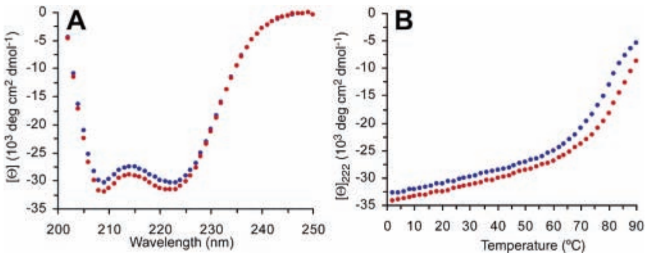

Figure 3. CD data for single Glu/Glu mismatch complexes. Wavelength (A) and thermal denaturation (B) data for solutions of T$_9$K:T$_{16}$E:T$_{23}$E (parallel, red circles) and T$_9$K:T$_{16}$E^a:T$_{23}$E (antiparallel, blue circles). All solutions are 10 μM total peptide in PBS buffer (10 mM phosphate, pH 7.5, 150 mM NaCl). Wavelength spectra taken at 25 °C.

Having demonstrated the formation of well behaved coiled-coil trimers, we sought to specifically verify relative strand orientation in the designed complexes. For the single

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(4) Schnarr, N. A.; Kennan, A. J. *J. Am. Chem. Soc.* **2003**, *125*, 667−671.

(5) Schnarr, N. A.; Kennan, A. J. *J. Am. Chem. Soc.* **2004**, *126*, 14447−14451.

(6) Although the parallel assemblies are technically new, they differ from known systems only by double replacement of T$_9$K for T$_9$E and T$_{16}$E for T$_{16}$K, which should not alter their stability.

(7) See Supporting Information for additional details.

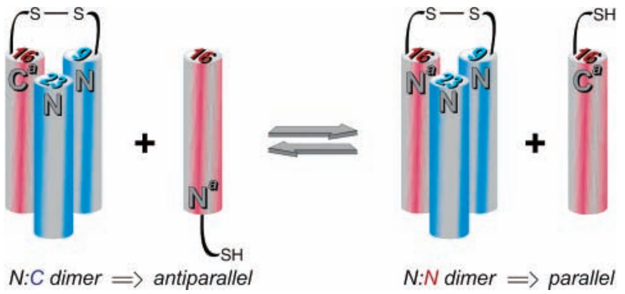

Figure 4. Disulfide exchange orientation assay schematic. Mixture of $T_{23}K$ with T_9K_{N-Cys} and $T_{16}E^a_{C-Cys}$ permits disulfide formation in antiparallel complex (left). Treatment with $T_{16}E^a_{N-Cys}$ allows exchange to a new disulfide in a parallel complex (right). Equilibrium populations of respective disulfides indicate orientation preference. Red and blue stripes indicate glutamic acid and lysine, respectively, at *e*/*g* interfaces. Numbers indicate cyclohexylalanine position.[8]

Lys/Lys mismatch structure, a disulfide exchange assay was employed (Figure 4), using peptides bearing Gly-Gly-Cys sequences at the N-terminus of T_9K (T_9K_{N-Cys}) and the N- and C-termini of $T_{16}E^a$ ($T_{16}E^a_{N-Cys}$, $T_{16}E^a_{C-Cys}$). In the antiparallel complex, the N-terminus of T_9K is close to the C-terminus of $T_{16}E^a$, implying that a T_9K_{N-Cys}:$T_{16}E^a_{C-Cys}$ disulfide (N:C) should be able to form such a structure. In contrast, a T_9K_{N-Cys}:$T_{16}E^a_{N-Cys}$ dimer (N:N) can only participate in a parallel arrangement. Under equilibrating conditions, the preference for one disulfide over the other reflects the relative trimer stabilities.[9]

Addition of $T_{16}E^a_{N-Cys}$ to an equimolar N:C/$T_{23}K$ solution results in little disulfide exchange, supporting the preference for an antiparallel trimer (Figure 5a). To ensure the intrinsic possibility of exchange the assay was repeated beginning with the preformed N:N dimer, which as expected rearranged to a similar final ratio (Figure 5b).

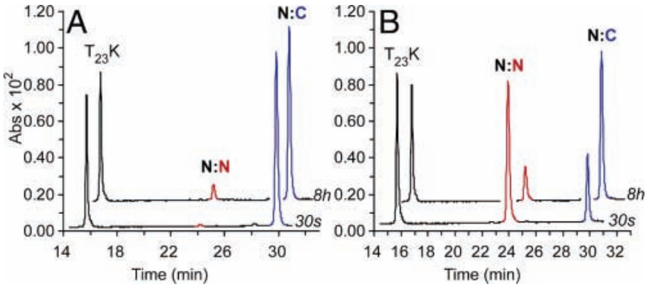

Figure 5. Disulfide exchange data for the antiparallel T_9K:$T_{16}E^a$:$T_{23}K$ complex (see Figure 4). HPLC traces taken after indicated intervals. (A) Initial N:C disulfide remains largely unchanged. (B) Initial N:N disulfide rearranges to N:C species which can form favored antiparallel complex.

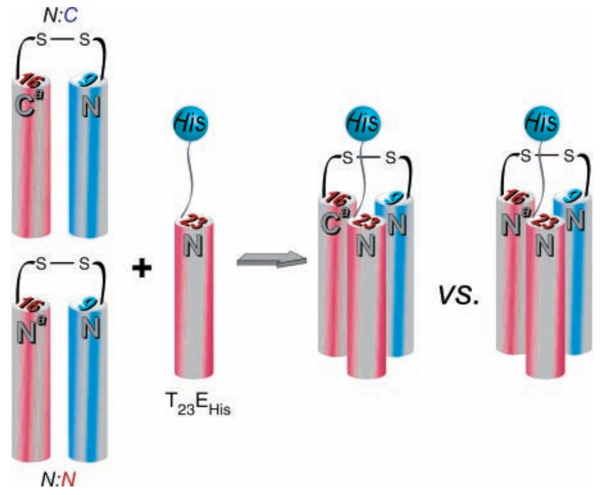

Figure 6. Combined disulfide/Ni-affinity competition assay schematic. Preformed disulfides between T_9K_{N-Cys}:$T_{16}E^a_{C-Cys}$ (N:C) and T_9K_{N-Cys}:$T_{16}E^a_{N-Cys}$ (N:N) can form antiparallel or parallel complexes, respectively, with $T_{23}E_{His}$. Analysis of the elution fraction from an initial equimolar mixture reveals relative orientation preference.[8]

Although disulfide exchange was effective in the single Lys/Lys case, its application to the Glu/Glu mismatched trimer is problematic. The latter complex is stable only at moderately low pH (below ∼6), whereas the exchange assay requires free thiolates and hence significantly higher pH. Accordingly, an alternative disulfide/nickel tag experiment was conducted. Attachment of a Gly-Gly-$(His)_6$ sequence to the N-terminus of $T_{23}E$ affords a derivative ($T_{23}E_{His}$) which binds to nickel-nitrilotriacetic acid (Ni-NTA) bearing agarose beads. We have shown that specific binding partners for tagged peptides are also retained.[3−5,10]

Application of the Ni-NTA method to orientation analysis involves direct competition between two preformed disulfides (Figure 6). The ratio of disulfides retained from an equimolar mixture of T_9K_{N-Cys}/$T_{16}E^a_{N-Cys}$ (N:N), T_9K_{N-Cys}/$T_{16}E^a_{C-Cys}$ (N:C), and $T_{23}E_{His}$ should reflect the parallel/antiparallel preference, since both compete for the limiting $T_{23}E_{His}$ peptide. The observed elution fraction contains largely the antiparallel components (N:C disulfide and $T_{23}E_{His}$), while the N:N dimer is in the supernatant.

After confirming the successful design of antiparallel heterotrimers with single-mismatch *e*/*g* interfaces, we undertook to exploit a unique feature of such structures. Since a Lys/Lys juxtaposition is tolerated at neutral to high pH, and a Glu/Glu arrangement is stable at low pH, the potential exists for altering trimer composition by pH manipulation. Given a initial high pH solution of a Lys/Lys mismatched trimer, reduction of pH and addition of a peptide capable of forming the now-favored Glu/Glu interface should result in a single specific strand exchange. We have previously

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[8] The helix depictions in Figure 4 are schematic and in particular do not reflect a likely register shift in the competing parallel complex.

[9] See refs 2d, 2f, and 5 for previous applications of this method.

[10] (a) Schnarr, N. A.; Kennan, A. J. *J. Am. Chem. Soc.* **2003**, *125*, 13046−13051. (b) Schnarr, N. A.; Kennan, A. J. *J. Am. Chem. Soc.* **2003**, *125*, 6364−6365.

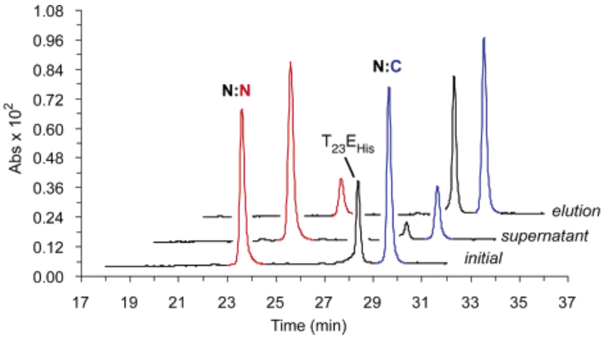

Figure 7. Disulfide/Ni-affinity competition data for single Glu/Glu mismatch complex. The initial solution is equimolar in $T_{23}E_{His}$, $T_9K_{N-Cys}/T_{16}E^a{}_{C-Cys}$ (N:C), and $T_9K_{N-Cys}/T_{16}E^a{}_{N-Cys}$ (N:N). The N:N dimer remains largely in the supernatant after mixing with Ni-NTA beads, while the N:C disulfide dominates the elution fraction. See Figure 6 for schematic representation

demonstrated this behavior in parallel systems; here it can be used for dynamic switching between parallel and antiparallel complexes.[10]

Analysis of the exchange experiment was conducted by Ni-NTA methods. An equimolar T_9K:$T_{16}K$:$T_{23}E_{His}$ mixture (parallel trimer) at pH 9.3 was treated with approximately one equivalent of $T_{16}E^a$, and the pH was lowered (to 5.5).[11] Although a low pH favorable complex could be made by replacement of either basic peptide, the need to retain core steric matching leaves $T_{16}K$ as the only candidate for substitution. HPLC analysis reveals, as expected, that the supernatant fraction contains the ejected $T_{16}K$ peptide, while the new antiparallel trimer components dominate the elution fraction (Figure 8). Similar results were obtained from an experiment in which the displaced peptide also bears the His tag, meaning that the antiparallel complex appears in the supernatant (Figure 9). In each case, successful pH-triggered conversion of a parallel coiled-coil heterotrimer to an antiparallel one has occurred.

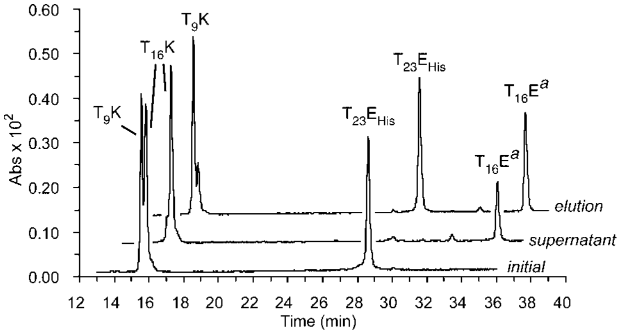

Figure 8. Ni-NTA analysis of orientation exchange. Initial solution (front) is equimolar T_9K:$T_{16}K$:$T_{23}E_{His}$ (one Lys/Lys, parallel complex, pH 9.3). Remaining traces reflect supernatant (middle) and elution (back) fractions after exposure to Ni-NTA beads, followed by addition of $T_{16}E^a$ and pH adjustment (to 5.5).

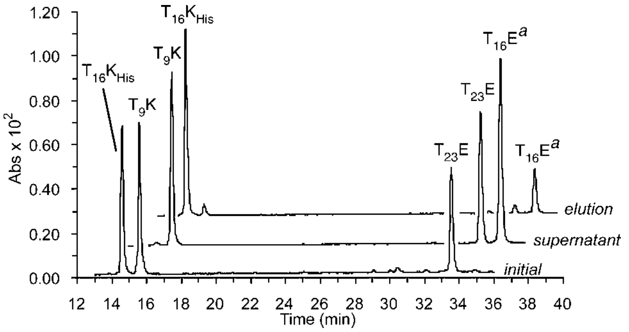

Figure 9. Ni affinity analysis of orientation exchange with displacement from resin beads. Initial solution (front trace) is equimolar T_9K:$T_{16}K_{His}$:$T_{23}E$ (one Lys/Lys, parallel complex, pH 9.3). Remaining traces reflect supernatant (middle) and elution (back) fractions after exposure to Ni-NTA beads, followed by addition of $T_{16}E^a$ and pH adjustment (to 5.5).

In summary, we have documented the feasibility of antiparallel coiled-coil trimers in which one of the three *e*/*g* surface contacts is electrostatically mismatched (Lys/Lys or Glu/Glu). The helicity and thermal stabilities of the new complexes are very similar to those of the more common parallel analogues, further buttressing design success. Finally, they are compatible with switching experiments that permit control of strand orientation by pH modulation. These results provide a foundation for the design of more sophisticated peptide assembly systems under dynamic external control.

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Supporting Information Available: Detailed experimental procedures and analytical ultracentrifugation data. This material is available free of charge via the Internet at http://pubs.acs.org.

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[11] Uncertainty regarding absolute resin loading results in a slight excess of $T_{16}E^a$.