Direct observation of the r(CUG) repeats in DM1 and MBNL1 aggregation complexes

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Myotonic dystrophy type 1 (DM1), the most common muscular dystrophy in adults, is caused by disorder expansion of a CTG repeat in the 3' untranslated region of the DM protein kinase (DMPK) gene. It is reported that patients have more than thousand repeats of CUG RNA are more likely to have severe symptom in DM1. Formation of the aggregation complexes between muscleblind like splicing regulator 1 (MBNL1) and the expanded CUG repeat RNA is considered to lead misregulated alternative splicing due to the lack of MBNL1. Because the interaction of the expanded CUG repeat RNA and MBNL1 could be a key step in the pathogeneses of DM1[1], [2], this interaction serves as a potential therapeutic target. Given the fact that the aggregates of the expanded CUG repeat RNA and MBNL1 have been observed *in vivo*, quantitative aspects for the formation of aggregation complexes, such as the effect of the number of the repeat of CUG for the aggregates formation, need to be further investigated.

The formation of MBNL1 complexes with short CUG repeat RNA was reported[3]. It is nonetheless important to characterize the aggregation complexes of the expanded CUG repeat RNA and MBNL1 *in vitro* by controlling the number of CUG repeat up to thousand to elucidate the molecular mechanism of RNA-mediated toxicity in DM1. In this study, DNA origami was utilized to mimic the 1000 repeats of CUG *in vitro* by controlling the numbers and the spatial organization of $(CUG)_{28}$ RNA (Fig. 1). The characteristics of the assembly of CUG repeat RNA and its complex with MBNL1 were directly investigated by means of high speed AFM.

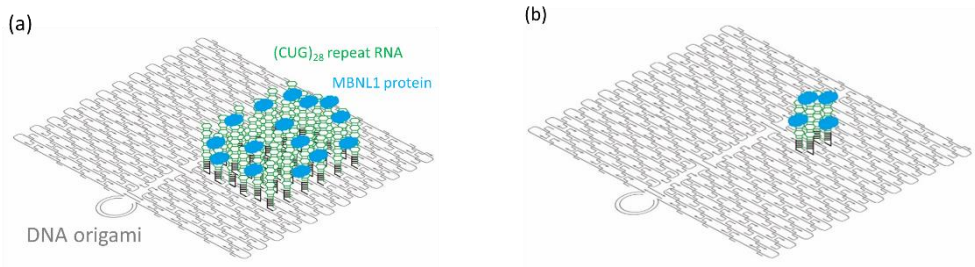

Fig 1. Design of a DNA nanostructure for assembling CUG repeat RNAs with (a) 36 $(CUG)_{28}$ RNA binding sites and (b) 4 $(CUG)_{28}$ RNA binding sites.

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