

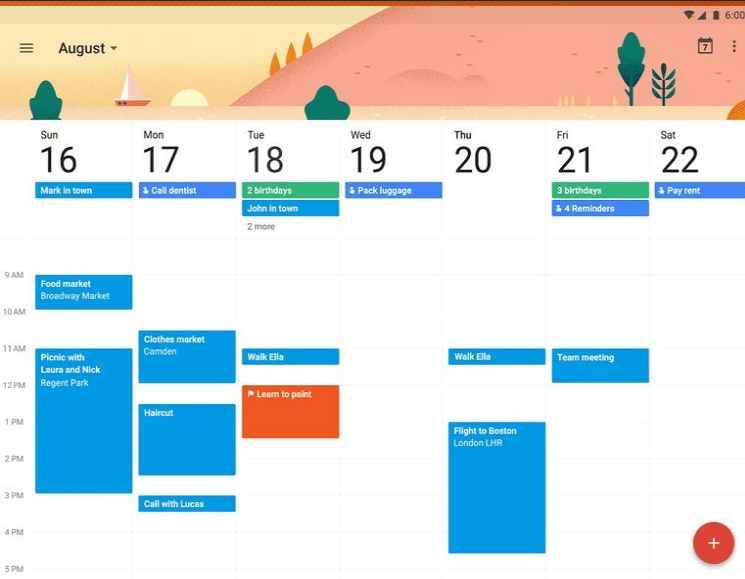
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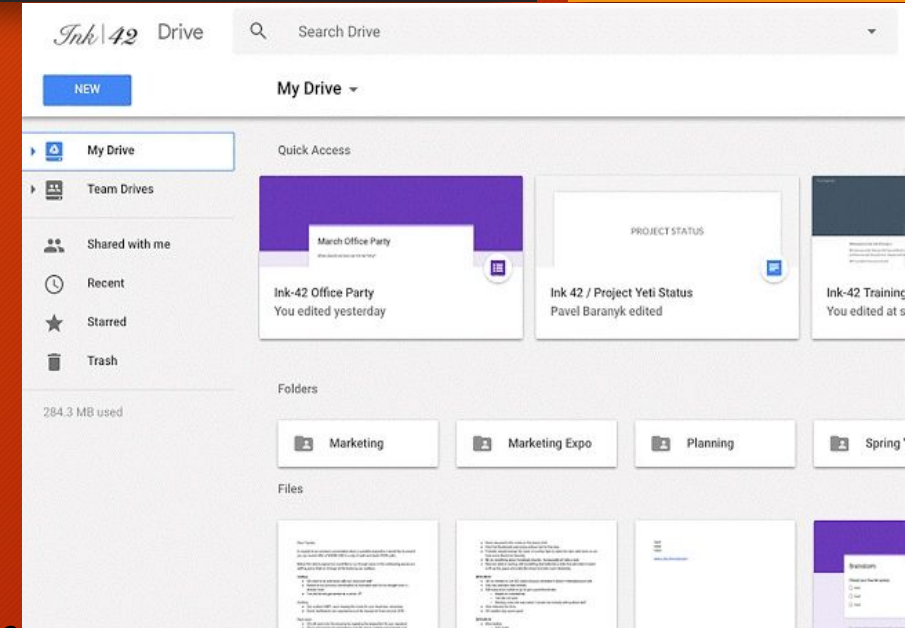
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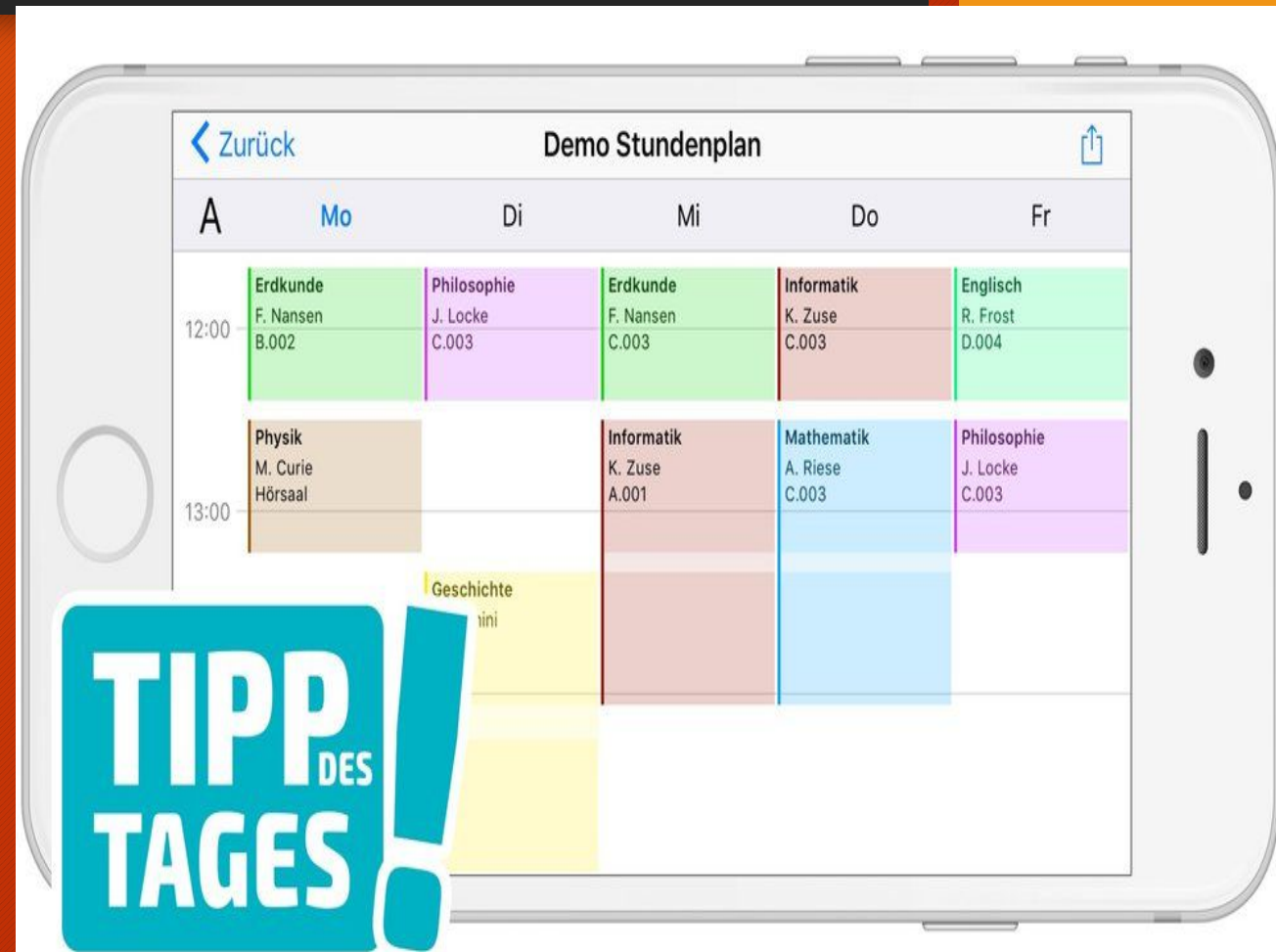
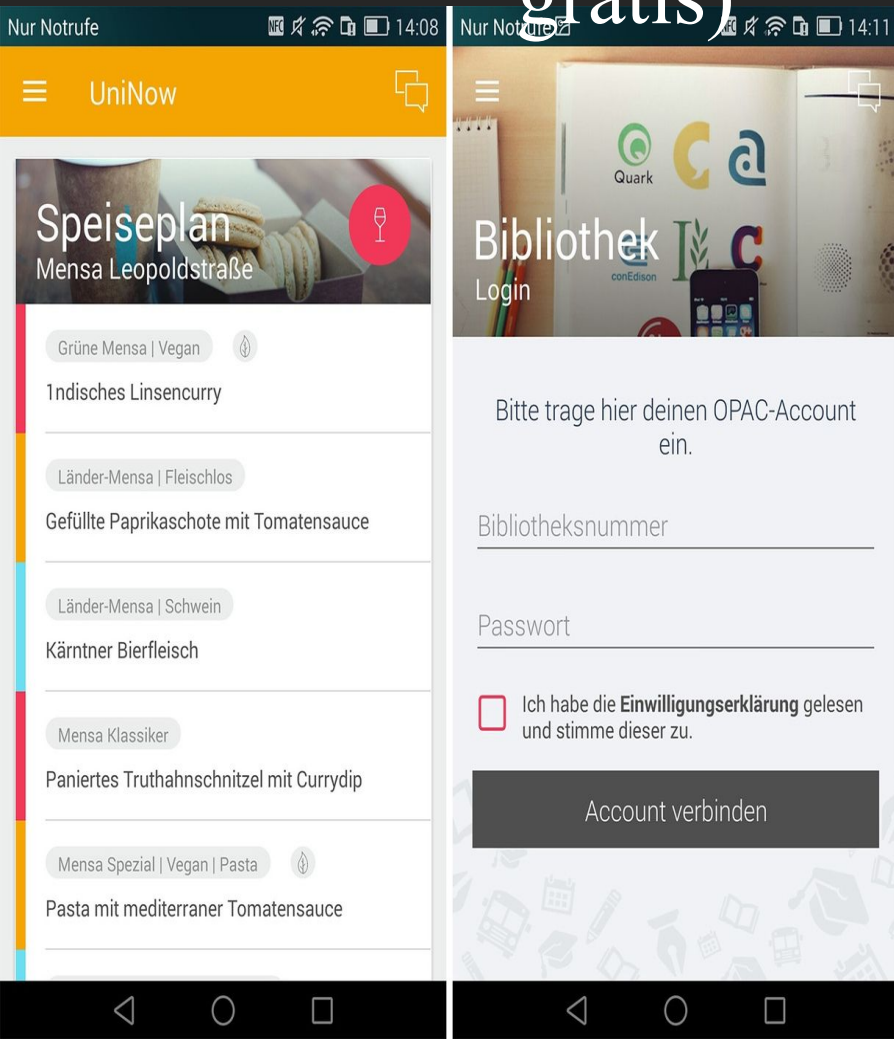
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A deoxyribozyme that synthesizes 2',5'-branched RNA with any branch-site nucleotide

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ABSTRACT

RNA molecules with internal 2',5'-branches are intermediates in RNA splicing, and branched RNAs have recently been proposed as retrotransposition intermediates. A broadly applicable *in vitro* synthetic route to branched RNA that does not require self-splicing introns or spliceosomes would substantially improve our ability to study biochemical processes that involve branched RNA. We recently described 7S11, a deoxyribozyme that was identified by *in vitro* selection and has general RNA branch-forming ability. However, an important restriction for 7S11 is that the branch-site RNA nucleotide must be a purine (A or G), because a pyrimidine (U or C) is not tolerated. Here, we describe the compact 6CE8 deoxyribozyme (selected using a 20 nt random region) that synthesizes 2',5'-branched RNA with any nucleotide at the branch site. The Mn²⁺-dependent branch-forming ligation reaction is between an internal branch-site 2'-hydroxyl nucleophile on one RNA substrate with a 5'-triphosphate on another RNA substrate. The preference for the branch-site nucleotide is U > C ≅ A > G, although all four nucleotides are tolerated with useful ligation rates. Nearly all other nucleotides elsewhere in both RNA substrates allow ligation activity, except that the sequence requirement for the RNA strand with the 5'-triphosphate is 5'-pppGAR, with 5'-pppGAR (R = purine) preferred. These characteristics permit 6CE8 to prepare branched RNAs of immunological interest, such as the proposed branched RNA intermediate of Ty1 retrotransposition. Because this

reaction. The ability to synthesize branched RNA of Ty1 retrotransposition to explore this important biochemical greater detail.

INTRODUCTION

RNA molecules with 2',5'-branched linkages are well-recognized intermediates in RNA splicing and group II introns and the spliceosomes of higher eukaryotes (1-3). More recently, branched RNAs have been proposed as intermediates in Ty1 retrotransposition, where they may play a key strand-transfer step (4,5), although recent work suggests that this hypothesis is likely to be incorrect (6). A general approach for preparing branched RNAs has been the investigation of such biochemical processes as the construction of topologically novel RNA structures (7-9) that incorporate covalently branched linkages. However, these methods have reported efforts toward 2',5'-branched RNA synthesis (10-14), but these methods are limited by the products that may be formed. In some solid-phase approaches, two of the strands from the branch site must have identical sequences. In addition, solid-phase RNA synthesis has an inherent limit of <100 nt (15,16), which is much shorter than the natural branched RNAs of biological interest. Phase synthesis of branched RNA requires splicing; therefore, this approach is not practical for many laboratories that lack either the facilities or the organic chemistry or familiarity with the procedures.

We have previously applied *in vitro* selection to identify deoxyribozymes (DNA enzymes) (17-20) that

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FluentU



Elisabeth
tz-Wiesn-Madl 2013

Der Abend , der war
wie ein Traum eigentlich
. Ich konnt 's gar nicht
glauben .

The evening, that was like a
dream actually. I couldn't
believe it.

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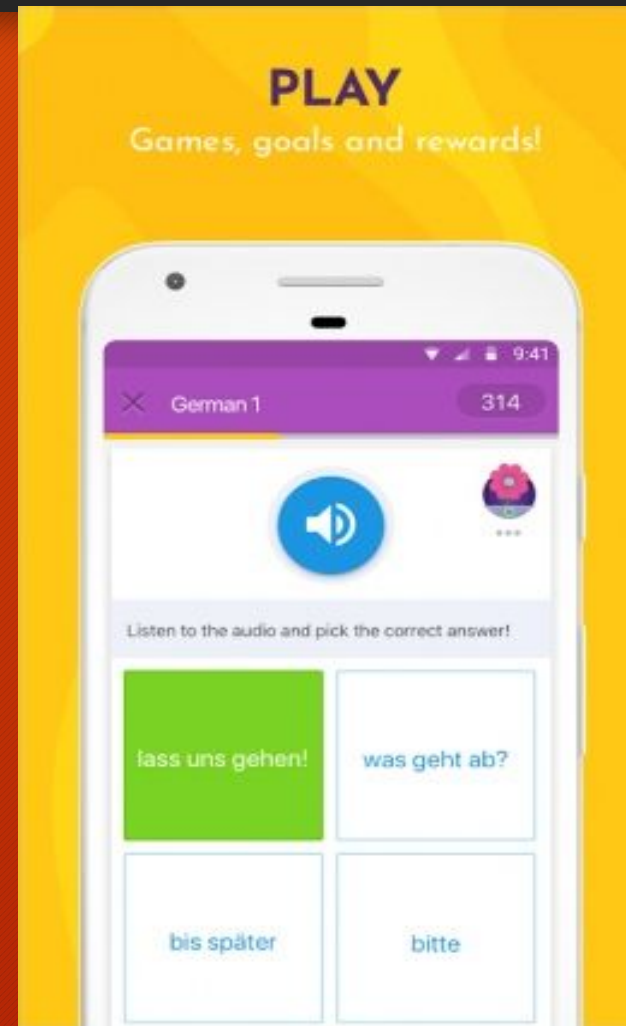
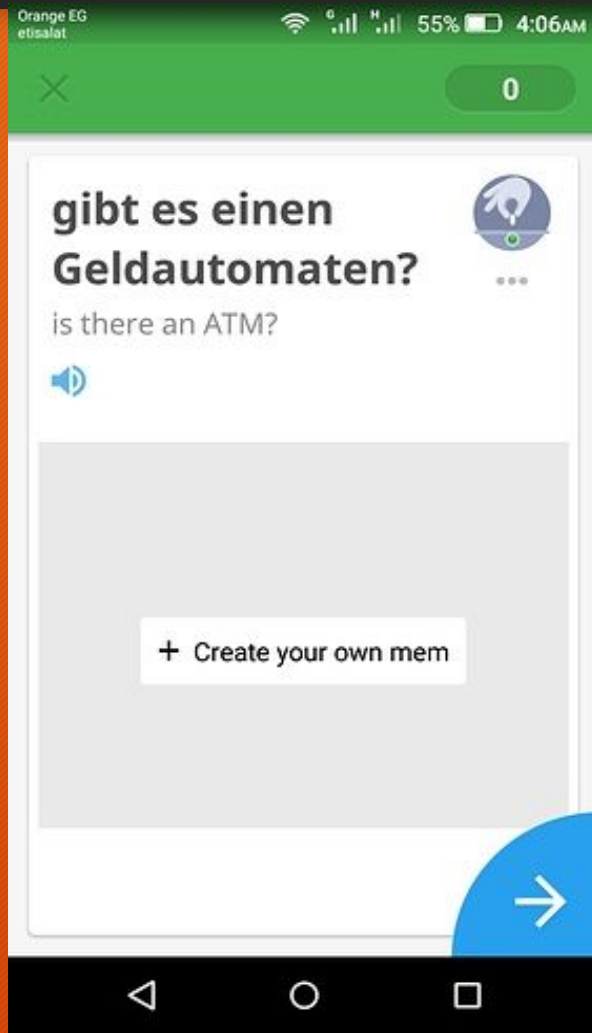
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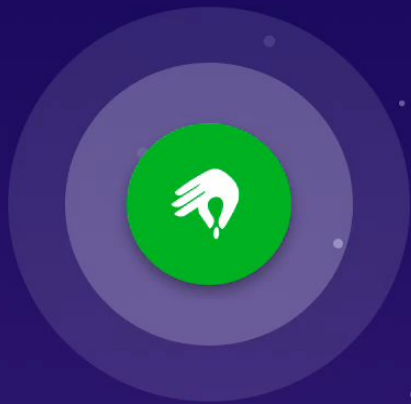


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