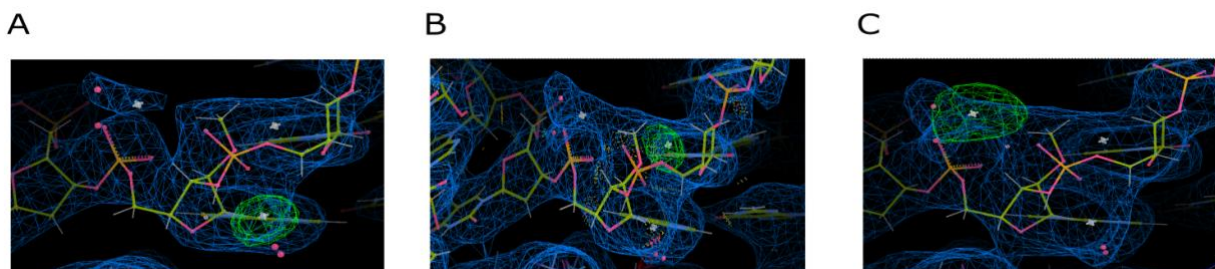
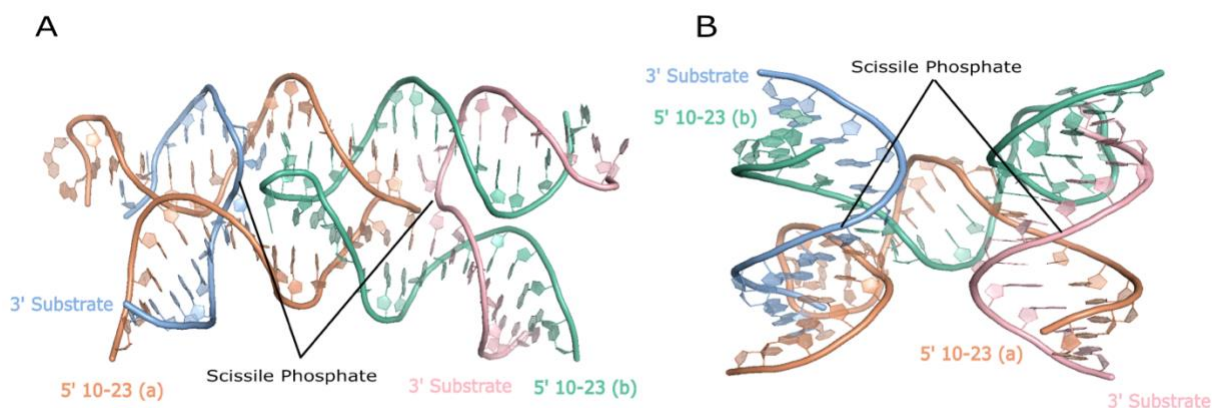


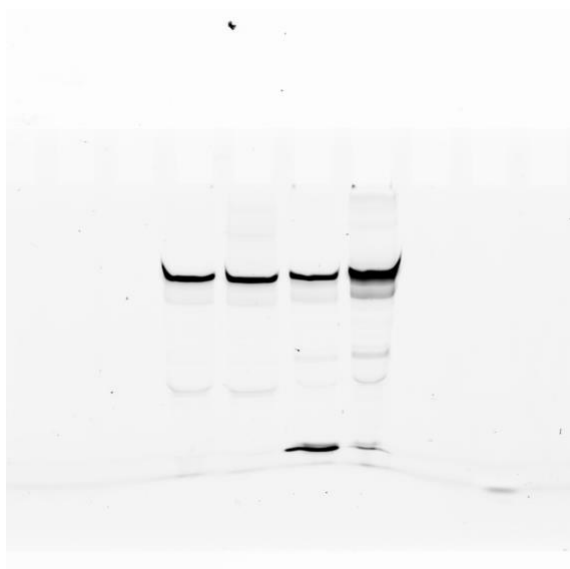
## Supplementary Figures:



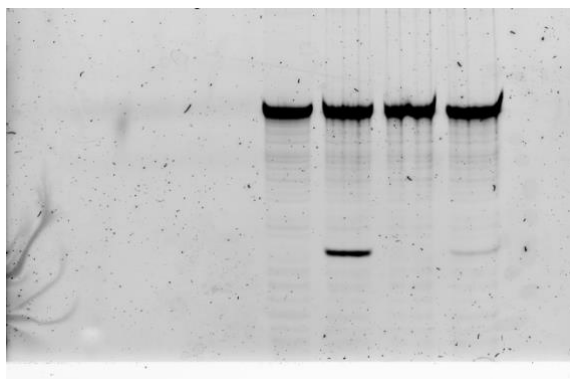
**Supplementary Figure 1:** Replacement of active site magnesium ions with sodium. Active site magnesium was replaced one by one with sodium and then run through a subsequent round of refinement. The positive peaks indicate the need for more electron density indicating that magnesium is appropriate at these sites. The replacements move from M1-3 (A-C respectively). FoFc  $\sigma$  = 4.0, 2FoFc  $\sigma$  = 1.0



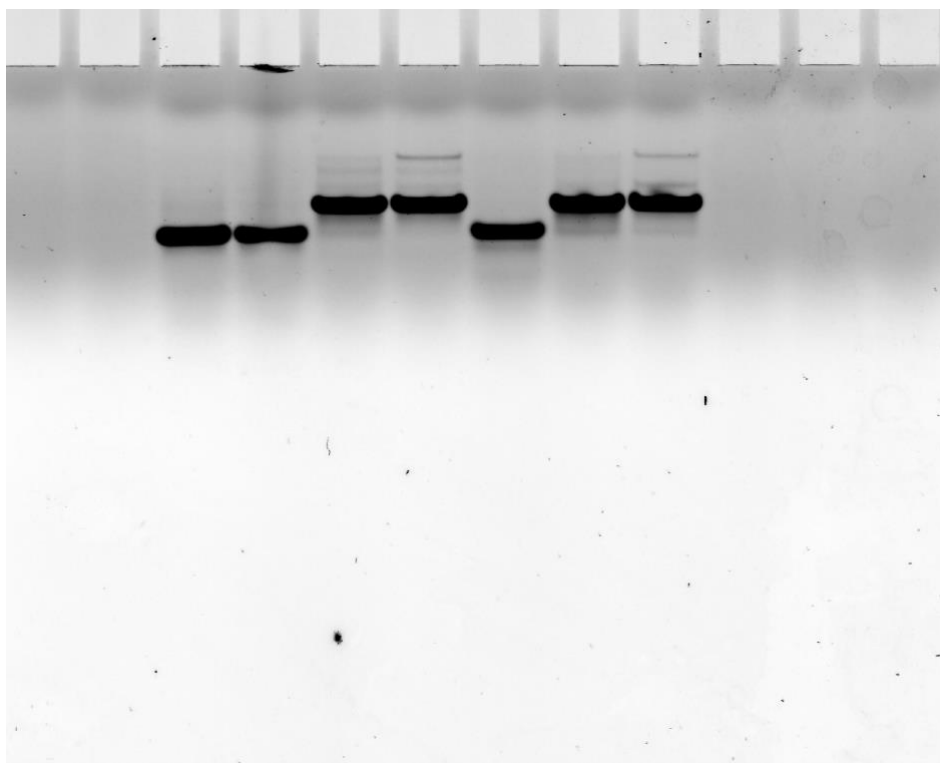
**Supplementary Figure 2:** Comparison to past 10-23 crystal structure. (A) current 10-23 DNAzyme crystal structure that showcases cis-binding of DNAzyme to substrate and a bend in the substrate strand at the catalytic core. (B) Past crystal structure of the 10-23 DNAzyme with trans-binding of the catalytic core to substrate and a linear substrate with no bending at the scissile phosphate.



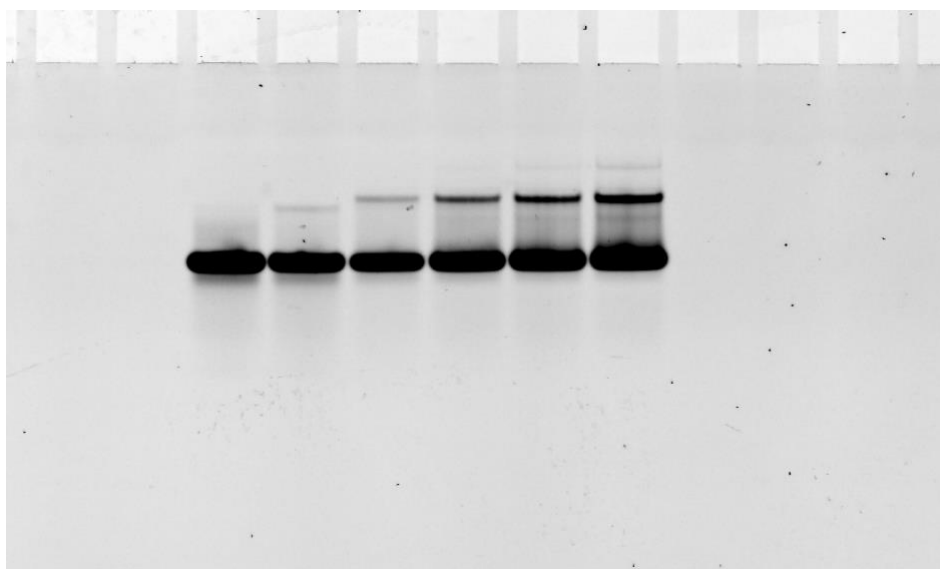
**Supplementary Figure 3:** Uncropped image of Figure 1B.



**Supplementary Figure 4:** Uncropped image of figure 3A.



**Supplementary Figure 5:** Uncropped image of Figure 3B. Additional three lanes the left are repeats of lanes 2-5 in Figure 3B but with the base pair adjacent to the cleavage site being an A-U base pair rather than the G-C base pair used for the structure.



**Supplementary Figure 6:** Uncropped image of Figure 3C.

## Supplementary Tables:

**Supplementary Table 1.** Data collection and refinement statistics (molecular replacement)

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|                                                      |                          |
|------------------------------------------------------|--------------------------|
| <b>Data collection</b>                               |                          |
| Space group                                          | C 1 2 1                  |
| Cell dimensions                                      |                          |
| <i>a</i> , <i>b</i> , <i>c</i> (Å)                   | 114.82, 48.41, 139.84    |
| $\alpha$ , $\beta$ , $\gamma$ (°)                    | 90, 90, 90               |
| Resolution (Å)                                       | 69.92-2.69 (2.79-2.69) * |
| <i>R</i> <sub>sym</sub> or <i>R</i> <sub>merge</sub> | 0.1147 (0.5649)          |
| <i>I</i> / $\sigma$ <i>I</i>                         | 2.16 (0.57)              |
| Completeness (%)                                     | 97.54 (83.55)            |
| Redundancy                                           | 2.0 (2.0)                |
| <b>Refinement</b>                                    |                          |
| Resolution (Å)                                       | 69.92-2.69               |
| No. reflections                                      | 42346                    |
| <i>R</i> <sub>work</sub> / <i>R</i> <sub>free</sub>  | 0.2528/0.2937            |
| No. atoms                                            | 4941                     |
| Macromolecules                                       | 4882                     |
| Ligand/ion                                           | 21                       |
| Water                                                | 38                       |
| <i>B</i> -factors                                    |                          |
| Macromolecules                                       | 71.28                    |
| Ligand/ion                                           | 83.52                    |
| Water                                                | 65.19                    |
| R.m.s. deviations                                    |                          |
| Bond lengths (Å)                                     | 0.007                    |
| Bond angles (°)                                      | 0.92                     |

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\*Values in parentheses are for highest-resolution shell.

**Supplementary Table 2:** List of all oligonucleotide sequences used in this study.

| Oligo Name          | Sequence                                                      |
|---------------------|---------------------------------------------------------------|
| 10-23 Xstal mA      | GCTGGGATmACATTGTGCGAAAGCACAAATGGGCTAGCTACAACGAATCCCAG<br>C    |
| 10-23 Xstal rA      | GCTGGGArUrArCAATCCTAGTTATAGGATTGGGCTAGCTACAACGAATCCCAG<br>C   |
| FAM10-23 DNAzyme    | FAM-CTCGCAGTATAGGCTAGCTACAACGAATCGCATAGG                      |
| 10-23 Substrate mA  | CCTATGCGAUmACATACTGCGAG                                       |
| 10-23 SnapBack      | TATCCGCGGAGACGCGGATAGGGCTAGCTACAACGAAGCCTATCGGAGACGA<br>TAGGC |
| 10-23 DNAzyme       | CTCGCAGTATAGGCTAGCTACAACGAATCGCATAGG                          |
| FAM 10-23 substrate | FAM-CCTATGCGArUrArCATACTGCGAG                                 |