|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | DBCOa  (mM) | DNA handle  (bp) | Buffer compositionb | Target moleculec (pM) | Conjugationd |
| Membrane protein scTMHC2 | 0.025 | 512 | PBS | 50 | O |
| 0.005% DDM, TBS | X |
| 0.1% DDM, TBS | X |
| 0.1% DDM, PBS | X |
| 0.1% DDM, 1% glycerol, PBS | X |
| 0.1 | PBS | 20 | O |
| 50 | O |
| 100 | O |
| 0.001% DDM, PBS | 100 | X |
| 0.1% DDM, PBS | 35 | X |
| 50 | X |
| 100 | X |
| 0.1% DDM, TBS | 5 | X |
| 10 | X |
| 20 | X |
| 40 | X |
| 50 | X |
| 0.1% CHAPSO, PBS | 100 | X |
| 1% glycerol, PBS | 100 | X |
| 1% bicelle (C), TBS | 20 | X |
| 40 | X |
| 1.5% bicelle (C), TBS | 10 | X |
| 20 | X |
| 40 | X |
| 1022 | PBS | 20 | O |
| 100 | O |
| 300 | O |
| TBS | 200 | O |
| 0.0001% DDM, PBS | 300 | O |
| 0.01% DDM, TBS | 200 | O |
| 0.05% DDM, TBS | 200 | O |
| 0.1% DDM, TBS | 300 | X |
| 0.1% DDM, PBS | 300 | X |
| 0.1% lyso PC (14:0), TBS | 150 | X |
| 0.5% bicelle (C), TBS | 150 | X |
| 200 | X |
| 1% bicelle (C), TBS | 150 | X |
| 300 | X |
| 1.5% bicelle (C), TBS | 150 | X |
| 2% bicelle (C), TBS | 150 | X |
| 300 | X |
| 2% bicelle (G), TBS | 150 | X |
| 1 | 512 | PBS | 100 | X |
| 2% bicelle (C), PBS | 100 | X |
| DNA hairpin 17S6L | 0.01 | 2011 | PBS | 20 | X |
| 50 | X |
| 100 | X |
| 0.025 | PBS | 50 | O |
| 100 | O |
| 0.1% DDM, PBS | 50 | O |
| 1.5% bicelle (C), PBS | 50 | X |
| 0.05 | PBS | 50 | O |
| 0.075 | 100 | O |
| 0.1 | 512 | 30 | X |
| 1024 | 30 | O |
| 2011 | 10 | O |
| 20 | O |
| 40 | O |
| 0.1% DDM, PBS | 20 | O |
| 40 | O |
| 1% bicelle (C), PBS | 100 | O |
| 1% bicelle (C), TBS | 40 | O |
| 1.5% bicelle (C), PBS | 100 | O |

Figure 3–source data 1. Full list of tested conditions for DBCO-azide conjugation. a DBCO concentration indicates the final concentration of DBCO-sulfo-NHS crosslinker of DBCO modification on bead surface. The other conditions (DNA handle length, buffer composition, and targe molecule concentration) indicate those in DBCO-azide conjugation step of the single-molecule system assembly. b PBS indicates a phosphate-buffered saline (0.1 M sodium phosphate, 150 mM NaCl, pH 7.3). TBS indicates a Tris-buffered saline (50 mM Tris-HCl, 150 mM NaCl, pH 7.5). The bicelle (C or G) consists of DMPC (or DMPG) lipid and CHAPSO detergent at a 2.5:1 molar ratio. The % indicates w/v %. c Final concentration of target molecule (membrane protein or DNA hairpin) for the surface tethering. d The symbol O indicates successful conjugation between DBCO-modified beads and target membrane proteins. In this case, specifically-tethered beads are found and the force-extension curves of the molecular constructs are obtained (confirmed by three replicates). The symbol X indicates unsuccessful conjugation or entire nonspecific binding of beads to the surface. In this case, no data are obtained. See Figure 3–source data 1 for the full list.