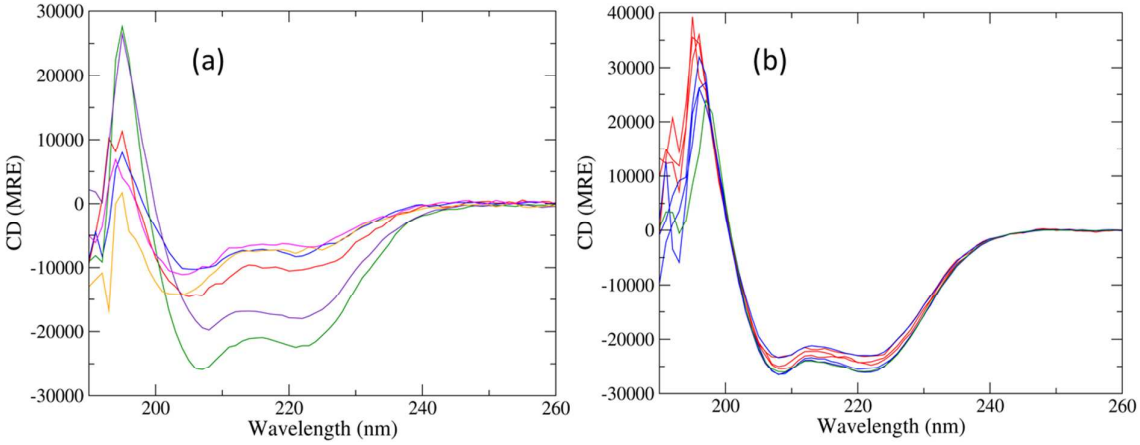
Supporting information for: *Construction of a chassis for a tripartite protein-based molecular motor*

Corresponding author Email address: e.h.c.bromley@durham.ac.uk

Section 1. Additional Circular Dichroism Data

Figure 1(a) shows the CD spectrum for each of the individual peptides, p1, p2, p3, p4, p5 and p6, in solution on their own. A small amount of homodimer formation is present in samples p3 and p4. Figure 1(b) shows the CD spectrum for combinations of the disulfide linked two domain peptides, including p6-1, p2-3 and p4-5 individually, mixtures of pairs including p6-1 with p2-3, p6-1 with p4-5 and p2-3 with p4-5, and finally the mixture of all 3 linked peptides.

Figure S1



(a) Individual CD spectra for the six Y hub peptides (1 to 6), each measured at 20 µM concentration in PBS buffer (pH 7.4), at 20 °C. The peptides 1 to 6 are shown in red, blue, green, purple, orange and magenta respectively. (b) CD data of the disulfide bonded peptides. The red spectra are the single disulfide bonded peptides, p6-1, p2-3 and p4-5, while the blue spectra are for the pairs of disulfide bonded peptides, p6-1 with p2-3, p2-3 with p4-5 and p4-5 with p6-1. The green spectrum is the mixture of all three disulfide bonded peptides

Section 2. Dynamic Light Scattering data

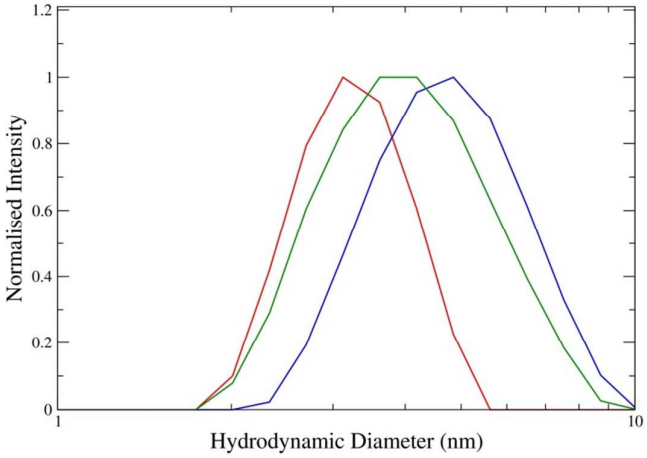
Dynamic light scattering data was collected on the designed coiled-coil pairs as well as the individual disulfide linked peptides and their pairwise combinations. Finally data was collected for the mixture of all three disulfide linked peptides.

Figure S2 shows the normalised intensity of the peaks of hydrodynamic diameter resulting from the DLS measurement. This indicates that the hydrodynamic diameter is between 2 and 3 nm for the designed coiled-coil pairs as expected.

Figure S3 shows the normalised intensity of the peaks of hydrodynamic diameter resulting from the DLS measurement of the individual disulfide linked peptides and their mixtures. These data indicate that the individual peptides have hydrodynamic diameters between 3 and 4 nanometers, which is

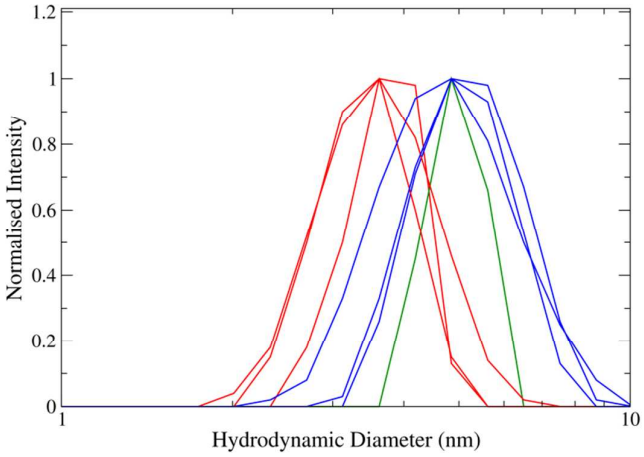
consistent with them having folded into intramolecular anti-parallel coiled coils. The pairs of linked peptides contain structures with hydrodynamic diameters between 4 and 6 nanometers. This is consistent with the formation of intermolecular dimers extending the full length of the peptides (7 or 8 heptad repeats). The mixture of all three linked peptides shows a peak centered on a hydrodynamic diameter of 5 nm. This peak has a much smaller width indicating a more compact or symmetric structure and is therefore consistent with the formation of the designed tripartite hub.

Figure S2



DLS data of the designed peptide pairs. Shown are representative measurements of p1,p2 (red), p3,p4 (blue) and p5,p6 (green)

Figure S3



DLS data of the disulphide-bonded peptides. The red peaks are representative measurements of the disulfide bonded peptides, p6-1, p2-3 and p4-5, while the blue peaks are the pairs of disulfide bonded peptides, (p6-1, p2-3), (p2-3, p4-5) and (p4-5, p6-1). The green peak is the mixture of all three disulfide bonded peptides.

Section 3. AUC fitting information

The values for $\bar{v}$, the partial specific volume of the protein, used in the AUC data fitting are given in tables S1 and S2.

Table S1.

Sample	Expected Molecular Weight / Da	Measured Molecular Weight / Da	$\bar{v}$
p1,p2	7041	7000 ± 1000	0.7567
p3,p4	7397	7100 ± 1000	0.7467
p5,p6	5816	5900 ± 1000	0.7640

Table S2

Sample	Expected Heterodimeric Molecular Weight / Da	Measured Molecular Weight / Da	$\bar{v}$
p6-1	6470	7300 ± 1000	0.746
p2-3	7096	9700 ± 1000	0.7482
p4-5	6682	7600 ± 1000	0.7458
p6-1, p2-3	13566	16000 ± 1000	0.7467
p2-3, p4-5	13778	14000 ± 1000	0.7467
p4-5, p6-1	13151	16000 ± 1000	0.7460
p6-1, p2-3, p4-5	20248	19000 ± 1000	0.7460

Section 4. MALDI Data

In this section we present the detailed information from the disulfide exchange experiment, showing the assignment of the peaks seen in the pre-rearrangement data figure in the paper.

Table S3

MALDI peak Mass	Species Assignment	Species Mass	Notes
5826.6	6-6	5826	Absent post-rearrangement
6469.5	p6-1	6470	
6567.8	p4-5 (M-114)	6568	Single residue deletion of p4-5, seen in MALDI of p4-5 alone
6681.9	p4-5	6682	
6704.4	p4-5 ($M+Na^+$)	6704	Single residue insertion of p4-5, seen in MALDI of p4-5 alone
6810.3	p4-5 (M+128)	6810	Single residue insertion of p4-5, seen in MALDI of p4-5 alone
7095.1	p2-3	7096	
7117.5	p2-3 ($M+Na^+$)	7118	
7224.0	p2-3 (M+128)	7224	Single residue insertion of p2-3, seen in MALDI of p2-3 alone
7356.4	unassigned	-	Unassigned contaminant seen in the p2-3 MALDI