

Supplemental Information

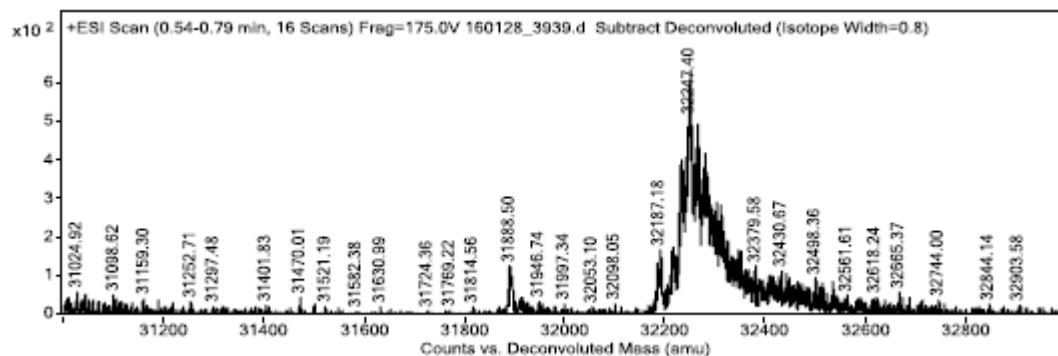


Figure SI 1. Mass spectrum of Hb-cyclooctyne (β -subunits cross-linked). ($(\beta$ -subunit (15867.22-1.01) \times 2) + $C_{26}O_7N_2H_{30}$ (482.58)) = 32215.00 g/mol. We cannot attribute the mass spectrum heterogeneity to an impurity of the cyclooctyne handle, since Hb-cyclooctyne reacts to completion in the presence of bis-azide (see Figure SI 3). The complex spectrum may result from transformations occurring during mass analysis.

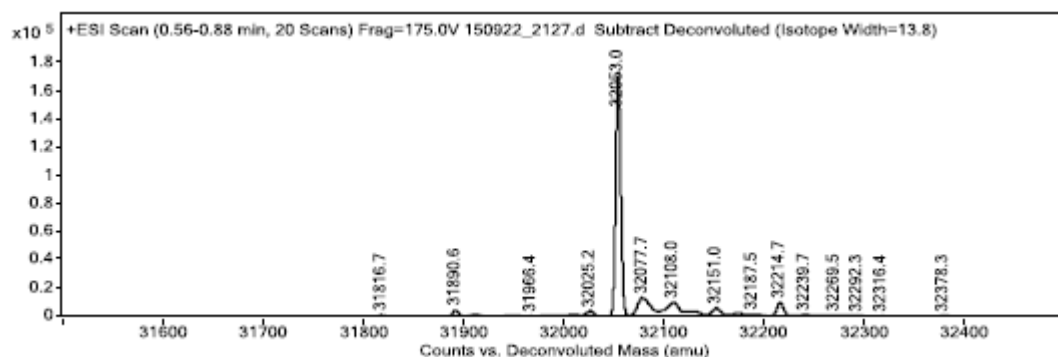


Figure SI 2. Mass spectrum of Hb-azide (β -subunits cross-linked). (β -subunits ((15867.22-1.01) \times 2) + $C_{17}O_3N_4H_{12}$ (320.33)) = 32052.75 g/mol.

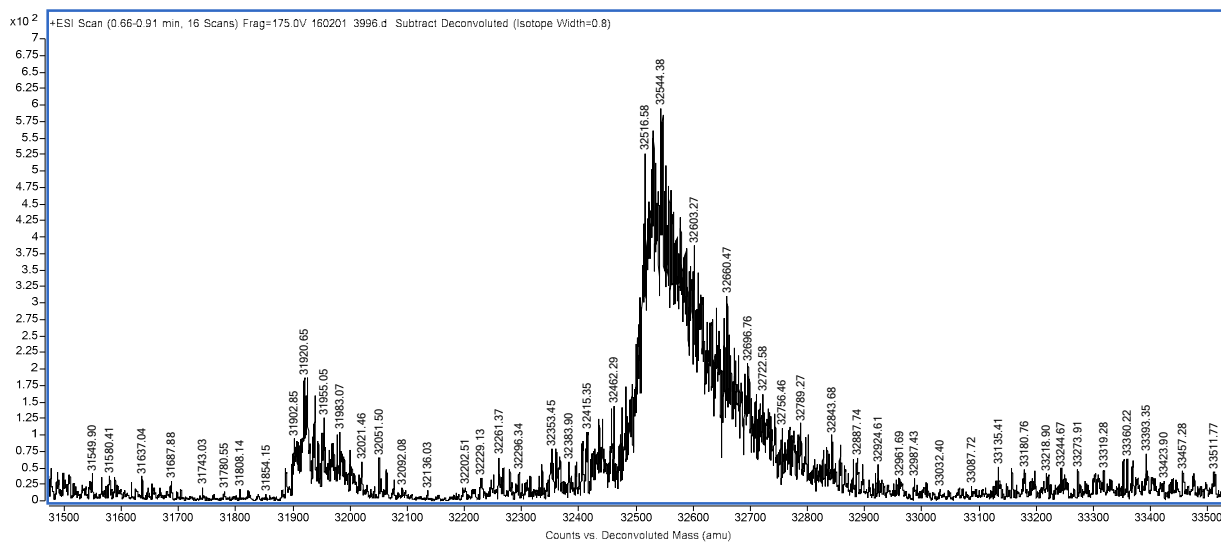


Figure SI 3. Mass spectrum of Hb-cyclooctyne (β -subunits cross-linked) modified with bis-azide (4,4'-diazidediphenylsulfone) only. ((β -subunit (15867.22-1.01) \times 2) + $C_{26}O_7N_2H_{30}$ (482.58) + $C_{12}SO_2N_6H_8$ (300.30)) = 32515.00 g/mol.

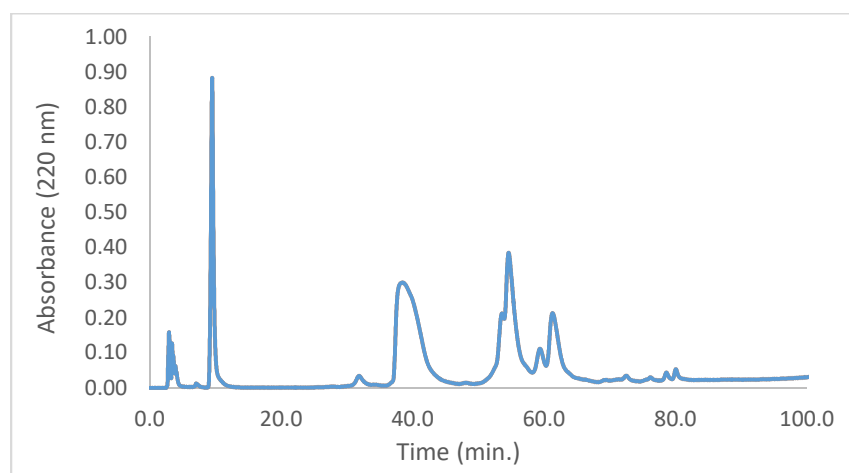


Figure SI 4. Reverse-phase HPLC of Hb-alkyne under dissociating conditions. Peaks are as follows: heme (10 min.); α -subunits (40 min.); β cross-linked subunits (alkyne modified, 54 min.).

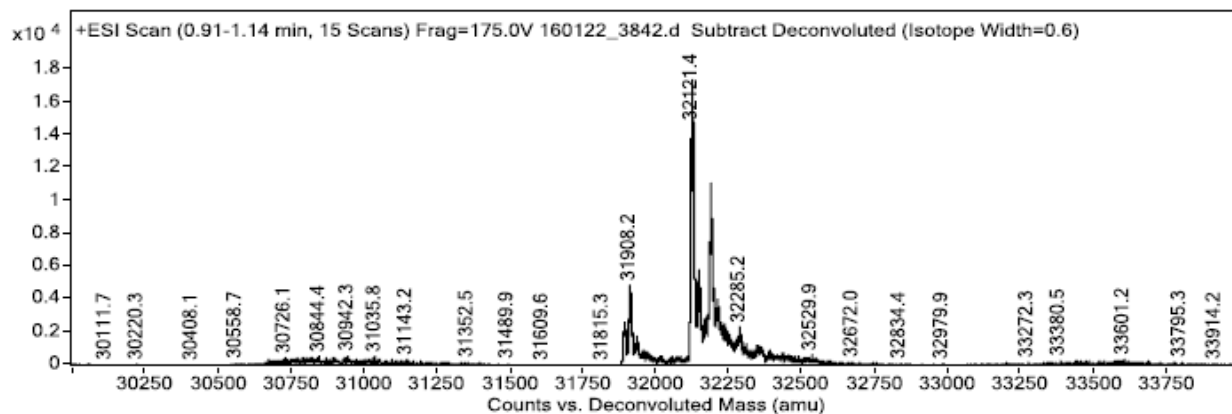


Figure SI 5. Mass spectrum of Hb-alkyne (β -subunits cross-linked). $((\beta\text{-subunits (15867.22-1.01)} \times 2) + \text{C}_{20}\text{O}_7\text{NH}_{23} (389.44)) = 32121.86 \text{ g/mol}$.

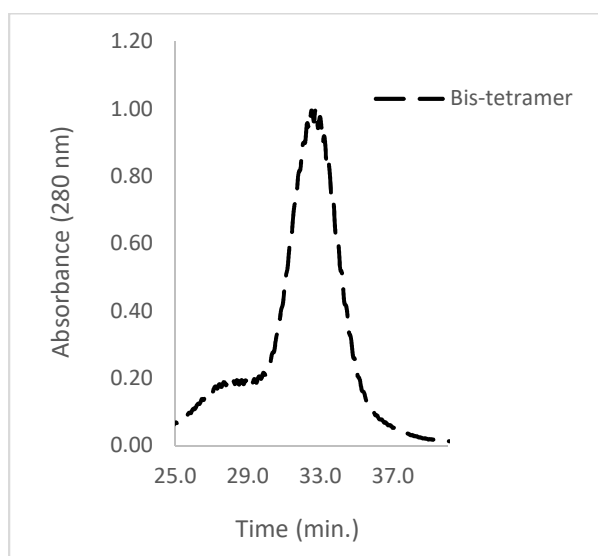


Figure SI 6. Size-exclusion HPLC trace of the purified bis-tetramer.

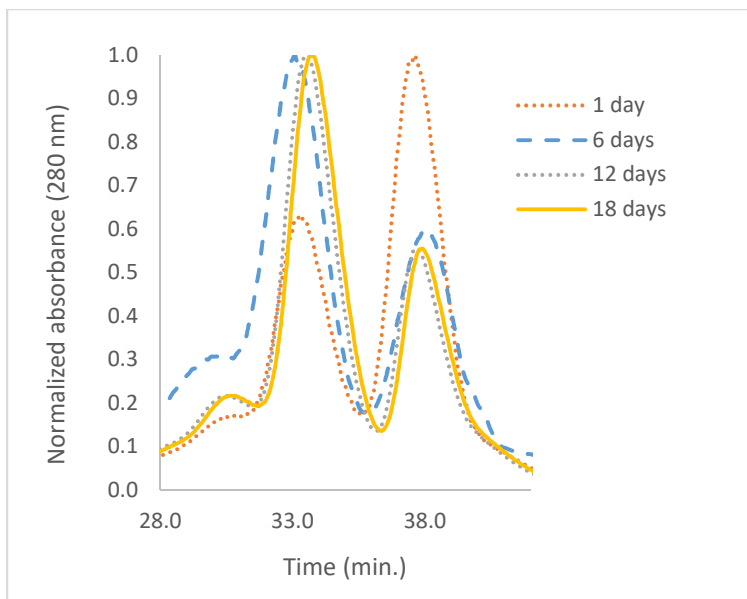


Figure SI 7. Size-exclusion HPLC trace of the products of the reaction of Hb-azide with Hb-cyclooctyne after 18 days.

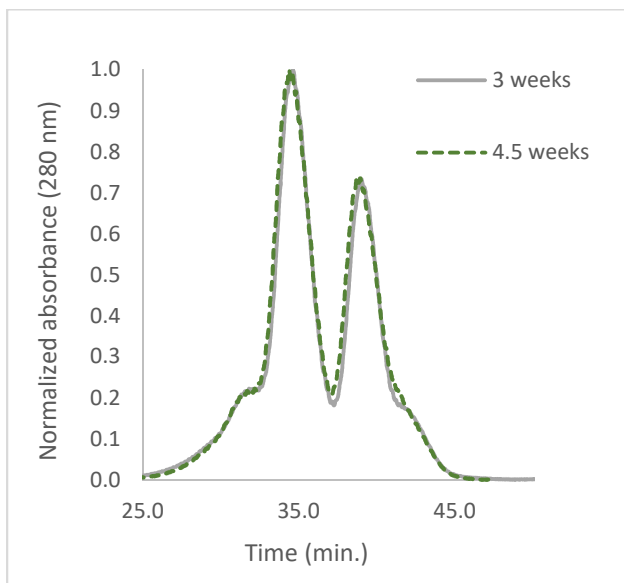


Figure SI 8. Size-exclusion HPLC trace of the products of the reaction of Hb-cyclooctyne with bis-azide (PEG spacer).