Supporting Information

Wheeldon *et al.* 10.1073/pnas.0805249105

SI Text

Construction of pQE9HSslac and Expression and Purification of HS-SLAC. The plasmid pSLAC was a kind gift from G. Canters (Leiden University, The Netherlands). The SLAC gene was extracted from pSLAC by PCR with the forward primer, GCA TAT GCATGC CG GCC GGG GGC GAG G and reverse primer CGC AAGCTT CTC CGG TTC CGC GGC GAC G. The upstream primer adds a unique SphI restriction site (underlined) and the downstream primer a unique HindIII site (underlined). The SLAC gene was ligated into pQE9AC10Acys (also a gift from D. Tirrell) at the unique SphI and HindIII sites. The resulting plasmid, pQE9HSslac, was propagated in a 5α *Escherichia coli* cell line (New England Biolabs), extracted and inserted into the SG13009 expression cell line.

Expression of HS-SLAC was done in 750-ml batches of TB media with 200 and 50 $\mu g \cdot ml^{-1}$ kanamycin inoculated with 10 ml of overnight culture of SG13009 containing pQE9HSslac. Cultures were grown to an OD = 1.4–1.5 at 30°C before induction with 0.5 mM IPTG. Expression continued for 18–20 h at 24°C. Cells were harvested by centrifugation at 15,000 $\times$ *g* for 15 min, resuspended in 50 ml of nickel-affinity chromatography buffer (20 mM imidazole, 20 mM Na_iP, 500 mM NaCl, pH 7.4) with EDTA-free protease inhibitor mixture (Roche). Cells were disrupted by sonication and a clarified lysate was produced after centrifugation at 15,000 $\times$ *g* for 30 min. Before purification the lysate was incubated with 1 mM $CuSO_4$ (Sigma), 20 units of DNaseI (New England Biolabs), and 20 μg of RNase A (Sigma).

Purification of HS-SLAC dimers requires nickel-affinity gel filtration chromatography. HS-SLAC elutes from a HisTrap Crude (GE Healthcare) column with 375 mM of imidazole. Active dimers of HS-SLAC elute from a HiLoad 16/60 Superdex 200 pg gel filtration column with 20 mM Na_iP and 150 mM NaCl, pH 7, 60 ml after sample injection. Purified samples were concentrated over 10-kDa cellulose filters (Amicon, Millipore) and stored at −20°C before use.

Amino Acid Sequence of HSH (with Histidine Side Chains in Italics)

MRGS HHHHHH GSDDDDKA SGDLENE VAQLERE VRSLEDE AAELEQK VSRLKNE IEDLKAE 60
IGDHVAPRDTSYRDPMG AGAGAGPEG AGAGAGPEG AGAGAGPEG AGAGAGPEG AGAGAGPEG 122
AGAGAGPEG AGAGAGPEG AGAGAGPEG AGAGAG-PEG AGAGAGPEG ARMPT SGDLENE 179
VAQLERE VRSLEDE AAELEQK VSRLKNE IEDLKAE IGD*H*VAPRDTSW 226

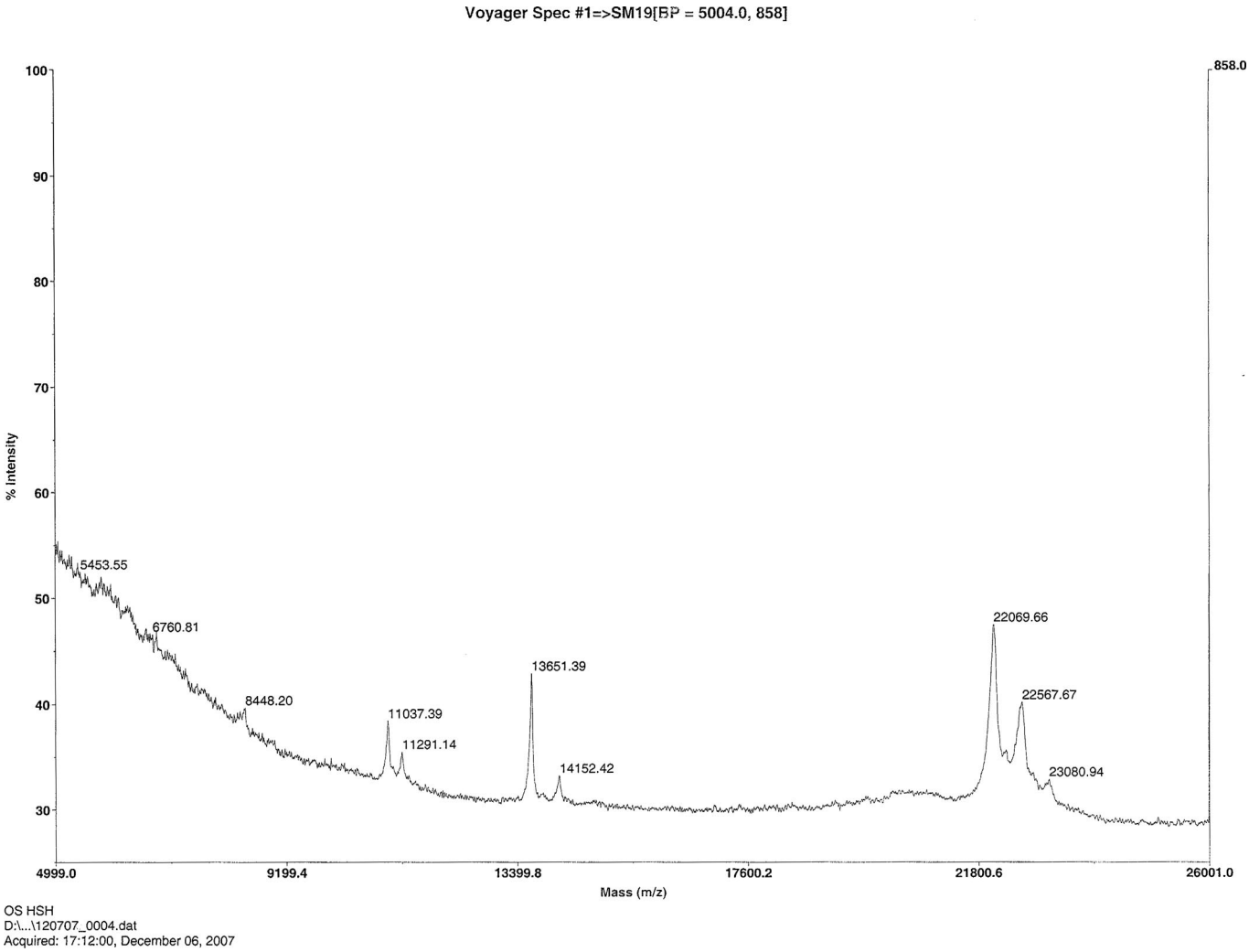

Fig. S1. MS MALDI-TOF spectra of OsHSH-1.

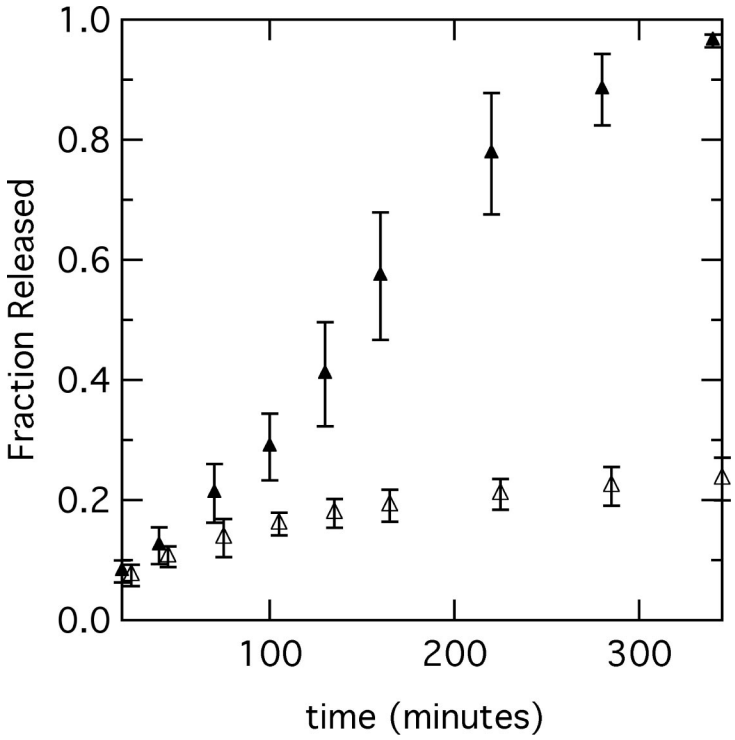

Fig. S2. Fractional release of 7.5 wt% hydrogels of OsHSH-1 with (closed) and without (open) glutaraldehyde cross-linking. Chemical cross-linking was achieved by subjecting rehydrated OsHSH hydrogels to a 1% glutaraldehyde solution for 5 min.

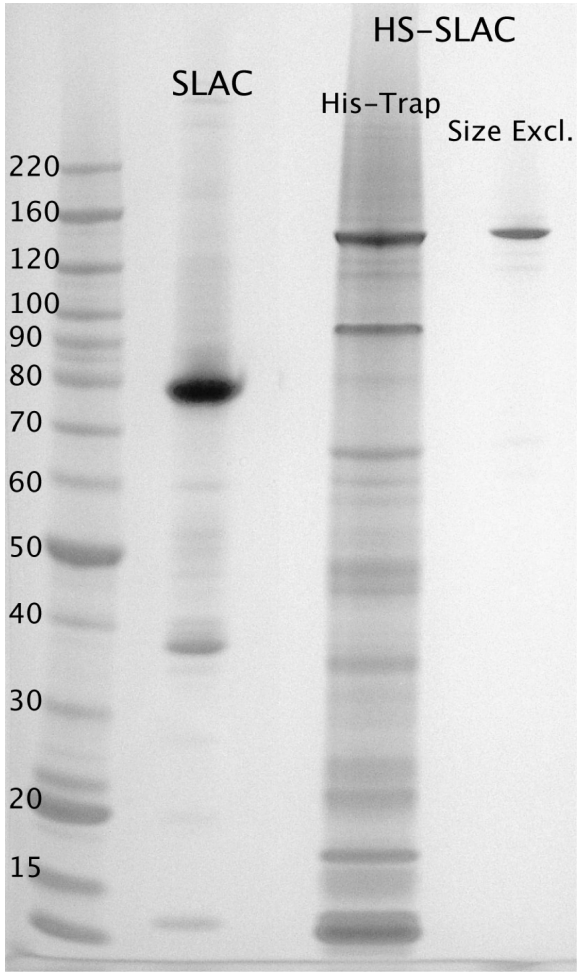

Fig. S3. 4–12% Bis-Tris SDS/PAGE of SLAC and HS-SLAC.

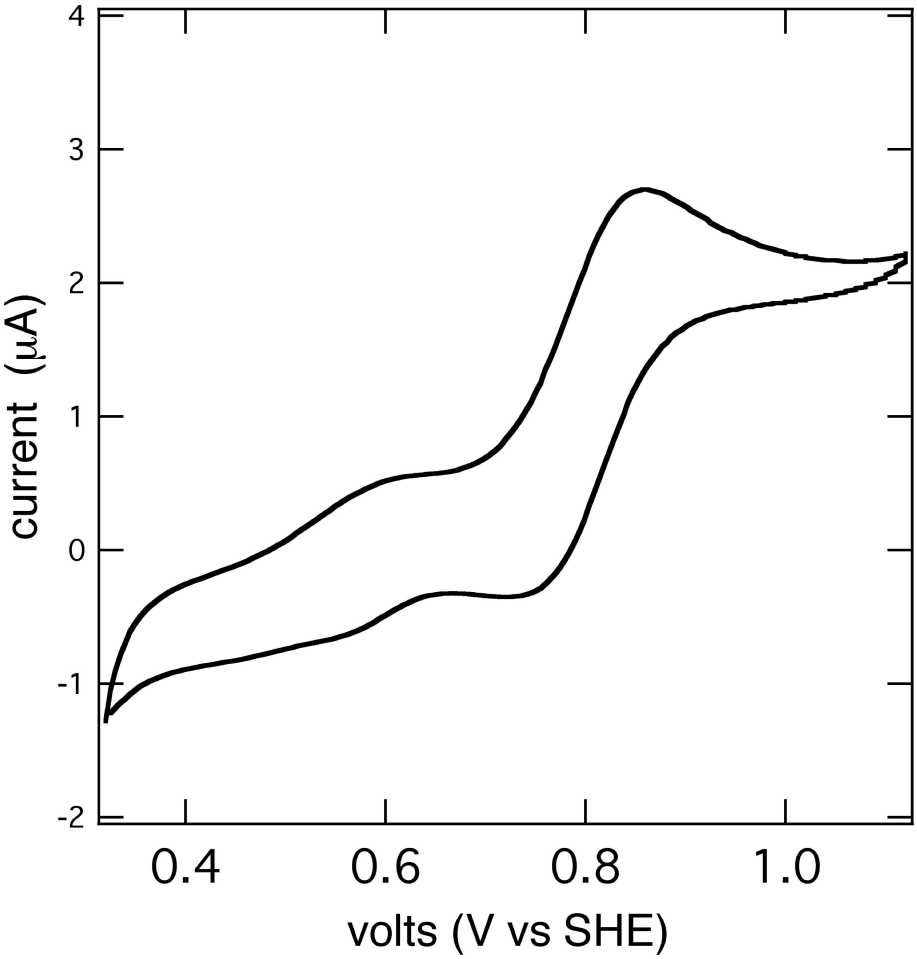

Fig. S4. Cyclic voltammogram of OsHSH-2 under N_2 atmosphere, 50 $mV \cdot s^{-1}$, 100 mM Na_iP, pH 7.0, at 25°C.

Table S1. MS-MALDI analysis of protease digested HSH modified with $[Os(bpy)_2Cl_2]Cl$

Peak	±	Protease	Possible assignments
1568.65	0.26	Trypsin	[Os(bpy)] + AEIGDHVAPR, nonspecific cleavage
1605.72	0.73	Trypsin	[Os(bpy)Cl] + AEIGDHVAPR, [Os(bpy)] + LKNEIEDLK
2292.84	0.81	Trypsin	[Os(bpy)Cl]Cl + GSHHHHHHG SDDDDK, nonspecific cleavage
2650.96	0.98	Trypsin	[Os(bpy)] + LKNEIEDLKAEIGDHVAPR
2686.63	–	Trypsin	[Os(bpy)Cl] + LKNEIEDLKAEIGDHVAPR, nonspecific cleavage
7424.87	2.53	Trypsin	[Os(bpy)2] + [Os(bpy)2Cl] + GSHHHHHHG. . .EIEDLK, nonspecific cleavage
1268.82	–	AspN	$[Os(bpy)_2Cl]Cl$ + DHVAPR
1995.28	–	AspN	$[Os(bpy)_2Cl]Cl$ + DLKAEIGDHVAPR
2051.87	–	AspN	$[Os(bpy)_2Cl]Cl$ + GSHHHHHHG SDDD
5962.37	–	AspN	$2[Os(bpy)_2]$ + GSHHHHHHGSDDDD. . .ELEQK