Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supporting Information for

Stabilization of bacterially expressed Erythropoietin by single site-specific introduction of short branched PEG chains at naturally occurring glycosylation sites

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1. Materials

CHO-EPO was purchased from Calichem; *p*-Azidophenylalanine was purchased by Bachem. The AlamarBlue® Reagent was from Life Technologies. Symmetrical PEG750-phosphites were synthesized according to published protocols.[1]

Plasmid and cell lines

The plasmid pEVOL-pAzF was a gift from Peter Schultz (Addgene plasmid #31186).[2] EPO gene sequences with an optimized *E.coli* codon usage were purchased from GeneArt. The sequence was cloned into pET11a (Novagen) using restriction sites BamHI/NdeI. The sequence of the EPO gene (without amber stop codons) is depicted below (restriction sites are underlined):

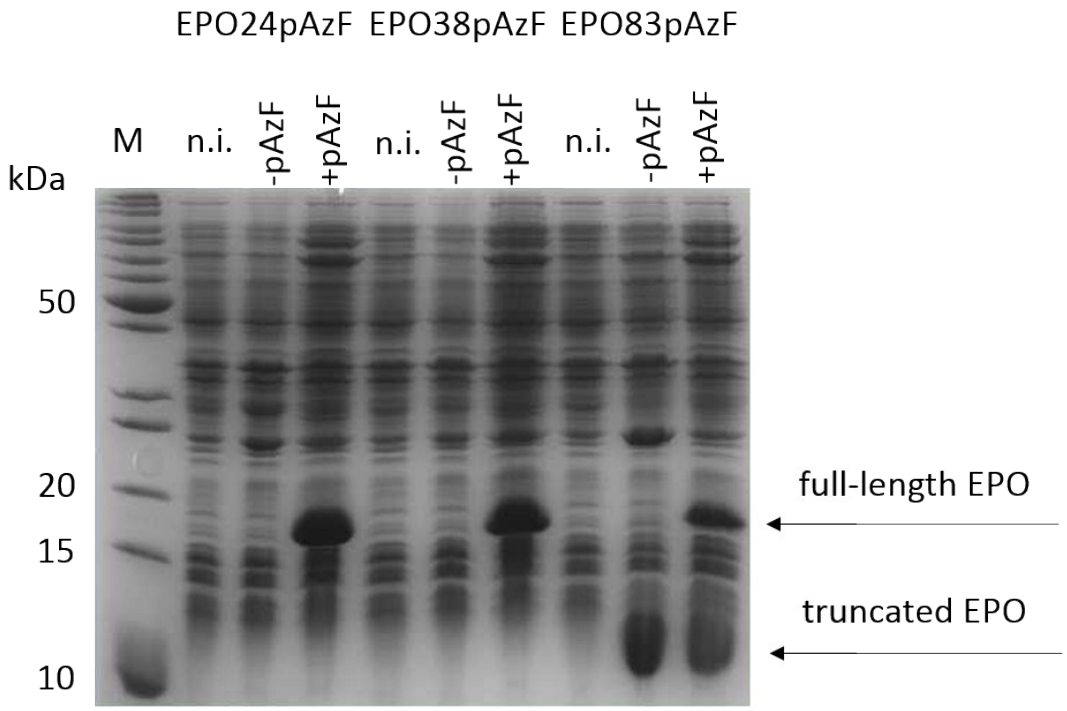
1 catatggcac cgcctcgtct gatttgtgat agccgtgttc tggaacgtta tctgctggaa
61 gcaaaagaag ccgaaaaaat taccaccggt tgtgcagaac attgtagcct gaatgaaaaa
121 attacagtgc cggataccaa agtgaatttt tatgcctgga aacgtatgga agttggtcag
181 caggcagttg aagtttggca gggtctggca ctgctgagcg aagcagttct gcgtggtcag
241 gcactgctgg ttaaaagcag ccagccgtgg gaaccgctgc agctgcatgt tgataaagca
301 gttagcggtc tgcgtagcct gaccaccctg ctgcgtgcac tgggtgccca gaaagaagca
361 atttctaata gcgatgcagc atctgcagca ccgctgcgta ccattaccgc agataccttt
421 cgtaaactgt ttcgcgtgta tagcaatttt ctgcgtggca aactgaaact gtataccggt
481 gaagcatgtc gtaccggtga tcgtcatcac catcatcatc attaaggatc c

The codons for Lys (AAA) in the box were singly mutated to the amber stop codon UAG.

The *E. coli* strain BL21(DE3) was used for the expression of all EPO variants.

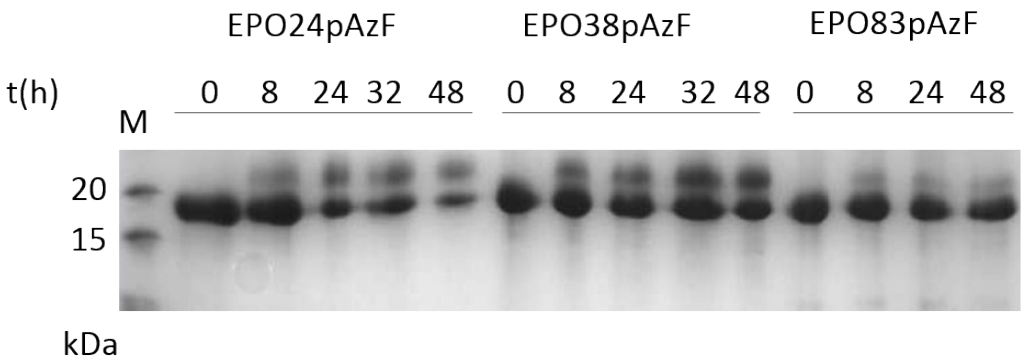
2. Supplementary Figures

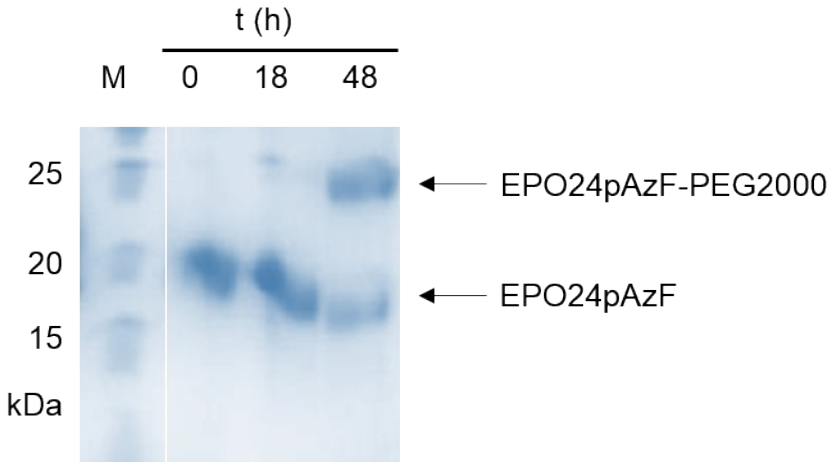
Supplementary Figure 1



Supplementary Figure 1. 15% SDS-PAGE. Expression profile of EPOpAzF in whole cell lysate. n.i.: not induced sample.

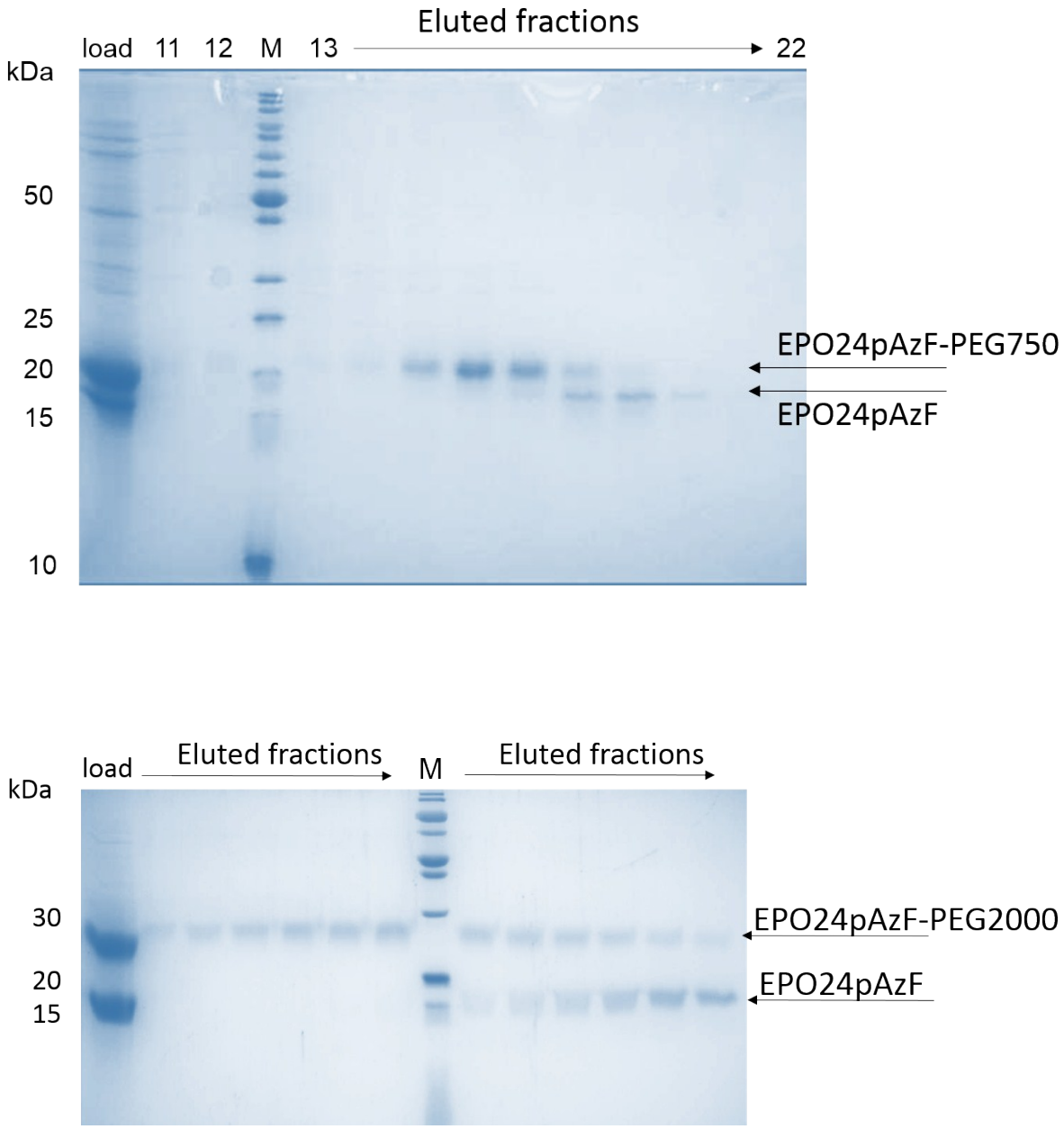
Supplementary Figure 2





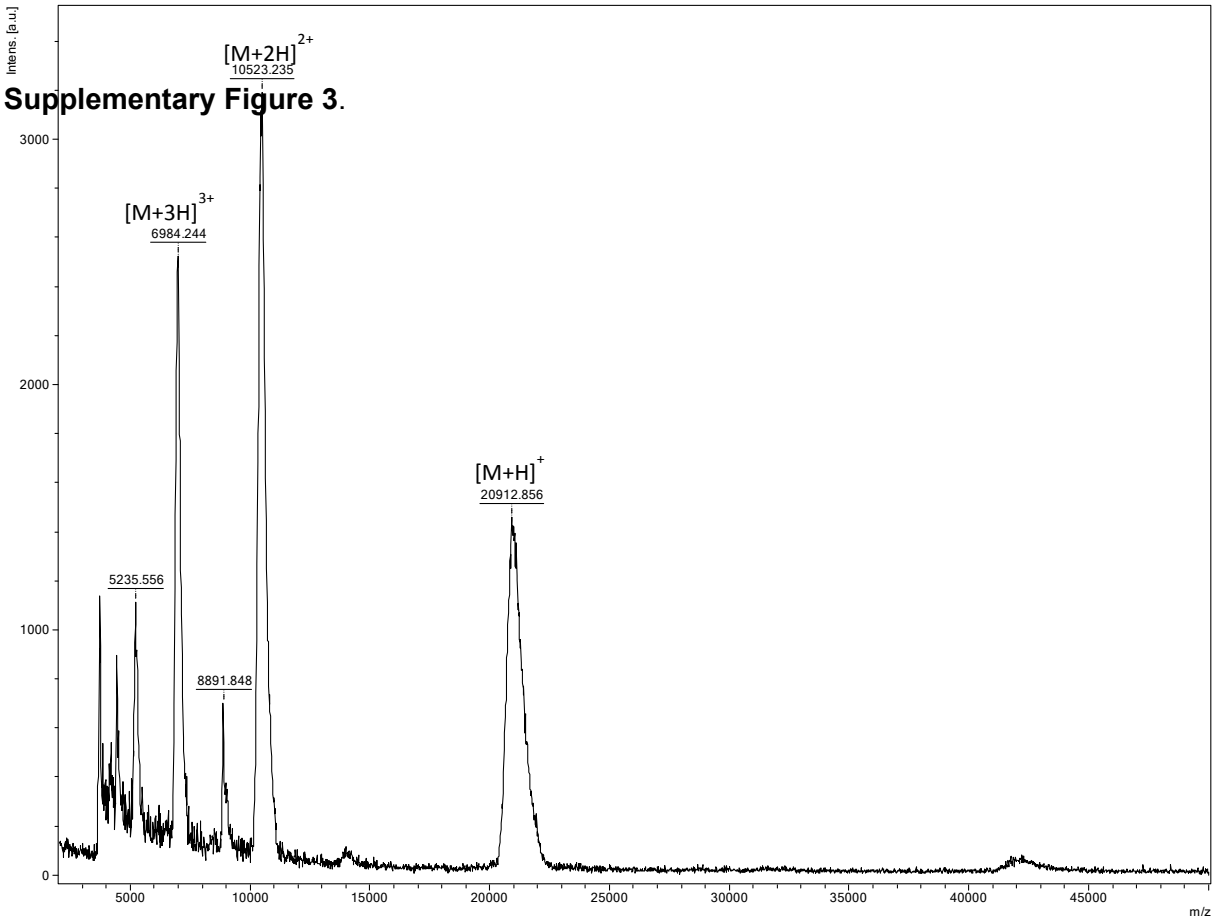
Supplementary Figure 2. 15% SDS PAGE showing the conversion of Staudinger-phosphite reaction between EPOpAzF and PEG750-phosphite (top) and EPO24pAzF and PEG2000-phosphite (bottom).

Supplementary Figure 3



Supplementary Figure 3. 15% SDS-PAGE showing the isolation of EPO24pAzF-PEG750 (top) and EPO24pAzF-PEG2000 (bottom) from the unPEGylated form after Gel filtration.

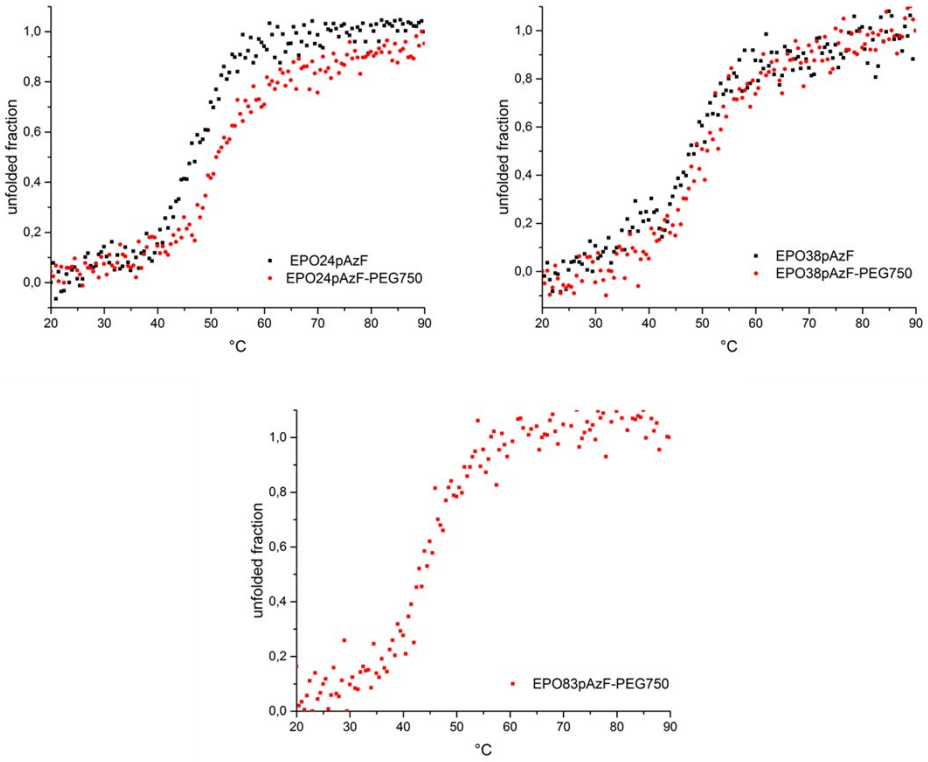
Supplementary Figure 4



Supplementary Figure 3.

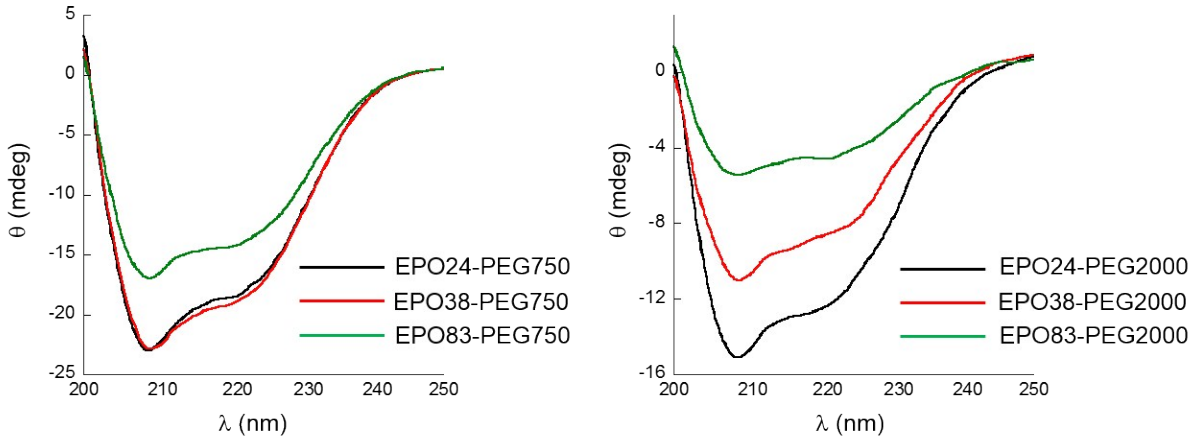
Supplementary Figure 4. MALDI-TOF Spectrum for PEGylated EPO24pAzF-PEG750

Supplementary Figure 5



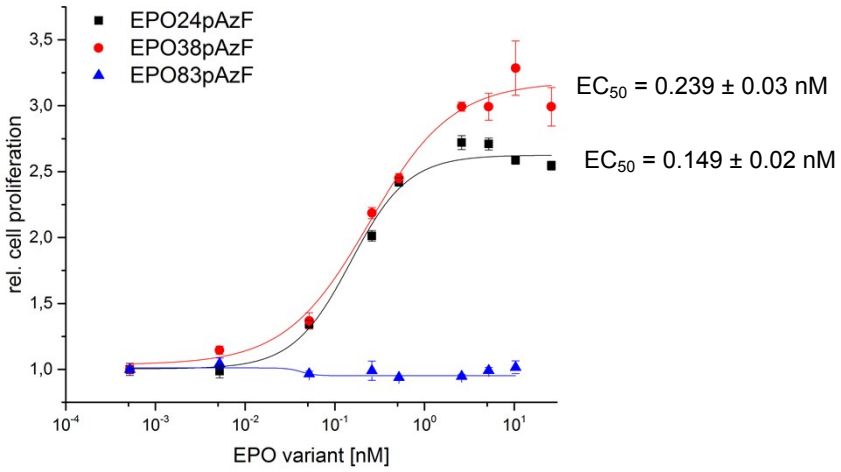
Supplementary Figure 5. Melting curves of EPO variants containing pAzF at different positions and their PEGylated analogs. Melting curves were recorded by following the decrease in the ellipticity at 220 nm during temperature increase. EPO83pAzF could not be isolated in sufficient amounts for characterization, due to extreme aggregation propensity.

Supplementary Figure 6



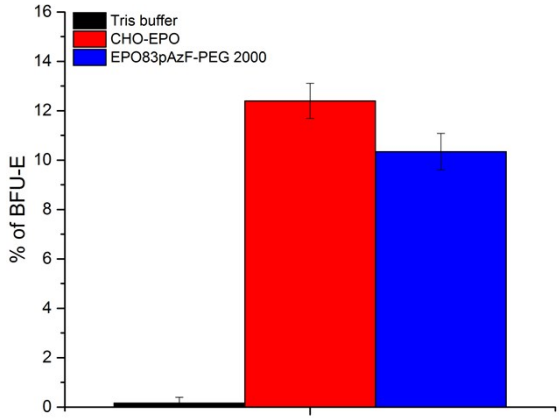
Supplementary Figure 6. Far-UV Circular Dichroism (CD) spectra of PEGylated EPO variants (original data)

Supplementary Figure 7



Supplementary Figure 7. *In vitro* bioactivity of EPO24pAzF, EPO38pAzF, and EPO83pAzF measured by cell proliferation assay with TF-1 cells. The relative increase in cell number was plotted against EPO concentration, and the data were fitted to a non-cooperative binding reaction with a single binding site (Hill coefficient = 1) (solid lines).

Supplementary Figure 8



Supplementary Figure 8. Percent of BFU-E colonies after treatment with 50ng/mL EPO variants on mouse bone marrow cells.

References

1. N. Nischan, A. Chakrabarti, R. A. Serwa, P. H. Bovee-Geurts, R. Brock and C. P. Hackenberger, *Angew Chem Int Ed Engl*, 2013, **52**, 11920-11924.
2. J. W. Chin, S. W. Santoro, A. B. Martin, D. S. King, L. Wang and P. G. Schultz, *J Am Chem Soc*, 2002, **124**, 9026-9027.