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Supplementary Materials for

B_{12}-induced reassembly of split photoreceptor protein enables photoresponsive hydrogels with tunable mechanics

Zhongguang Yang, Hong Kiu Francis Fok, Jiren Luo, Yang Yang, Ri Wang, Xinyu Huang, Fei Sun*

*Corresponding author. Email: kefsun@ust.hk

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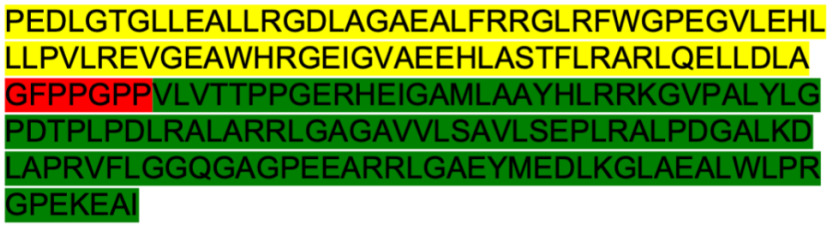


Fig. S1. Amino acid sequence of CarH$_C$. The N-terminal four-helix bundle, the loop region and the C-terminal B_{12}-binding domain are highlighted in yellow, red, and green, respectively.

CarH$_C$N (10.1 kDa)

GPGSEFPEDLGTGLLEALLRGDLAGAEALFRRGLRFWGPEGV
LEHLLLPVLREVGEAWHRGEIGVAEEHLASTFLRARLQELLDLA
GFPPGPP

CarH$_C$C (13.6 kDa)

GPGSEFPPGPPVLVTTPPGERHEIGAMLAAYHLRRKGVPALYL
GPDTPLPDLRALARRLGAGAVVLSAVLSEPLRALPDGALKDLA
PRVFLGGQGAGPEEARRLGAEYMEDLKGLAEALWLPRGPEK
EAI

Fig. S2. Amino acid sequences of CarH$_C$N and CarH$_C$C. The N-terminal His6-tag was removed by 3C protease before use in this study.

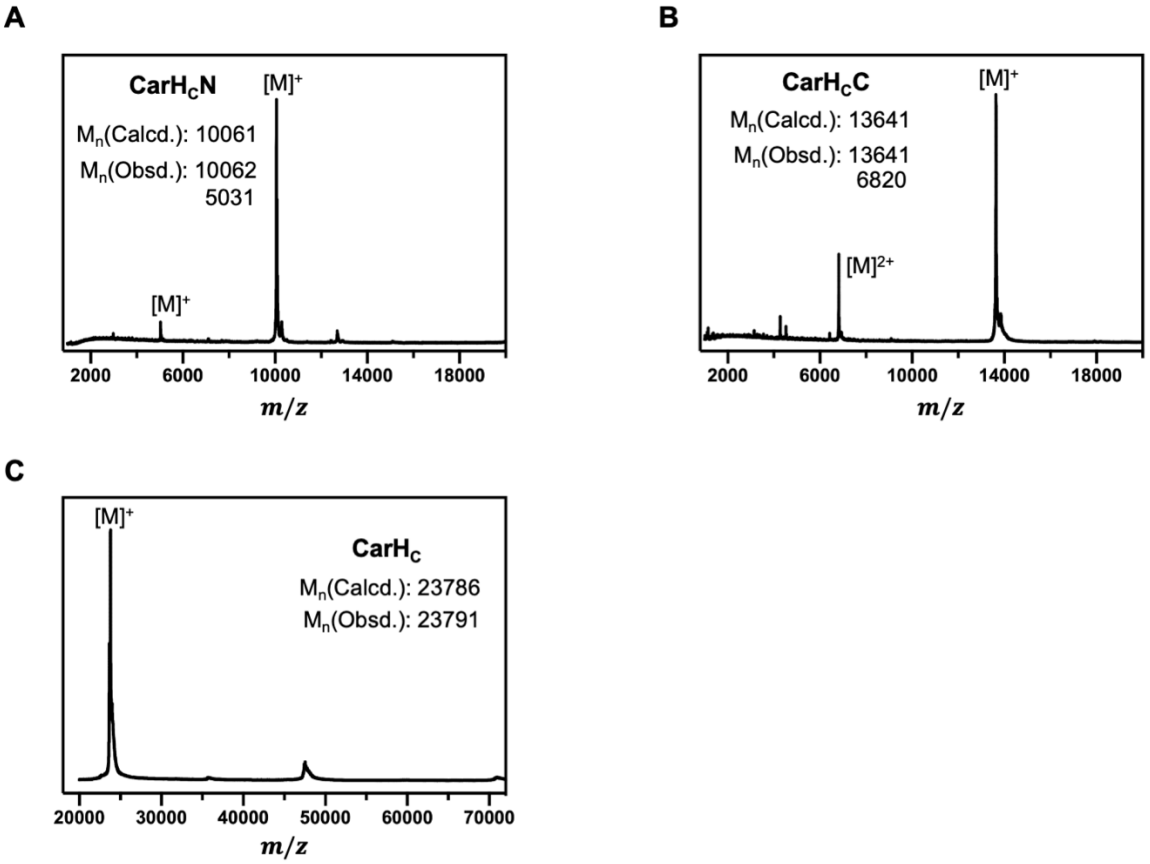


Fig. S3. MALDI-TOF mass spectra of (A) CarH$_C$N, (B) CarH$_C$C and (C) CarH$_C$.

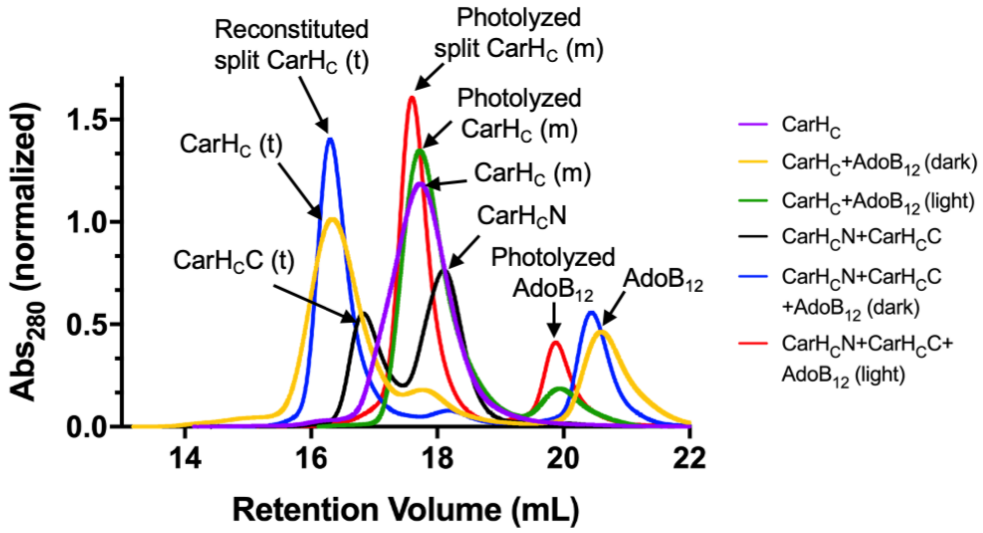
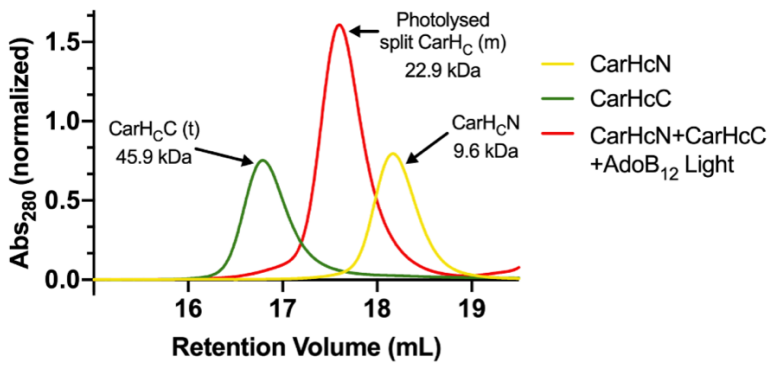


Fig. S4. SEC analyses of $CarH_C$ and split $CarH_C$ with addition of $AdoB_{12}$. "m" and "t" denote "monomer" and "tetramer", respectively.

A



B

Protein	Calculated MW	Measured MW
CarH$_C$N	10.1 kDa	9.6 ± 0.6 kDa
CarH$_C$C	13.6 kDa (monomer) 54.4 kDa (tetramer)	45.9 ± 0.6 kDa
Reconstituted split CarH$_C$	25.0 kDa	22.9 ± 0.3 kDa

Fig. S5. Size exclusion chromatography (SEC) (A) and dynamic light scattering (DLS) analyses (B) of CarH$_C$N, CarH$_C$C, and CarH$_C$N + CarH$_C$C + AdoB$_{12}$ (light). "m" and "t" denote "monomer" and "tetramer", respectively.

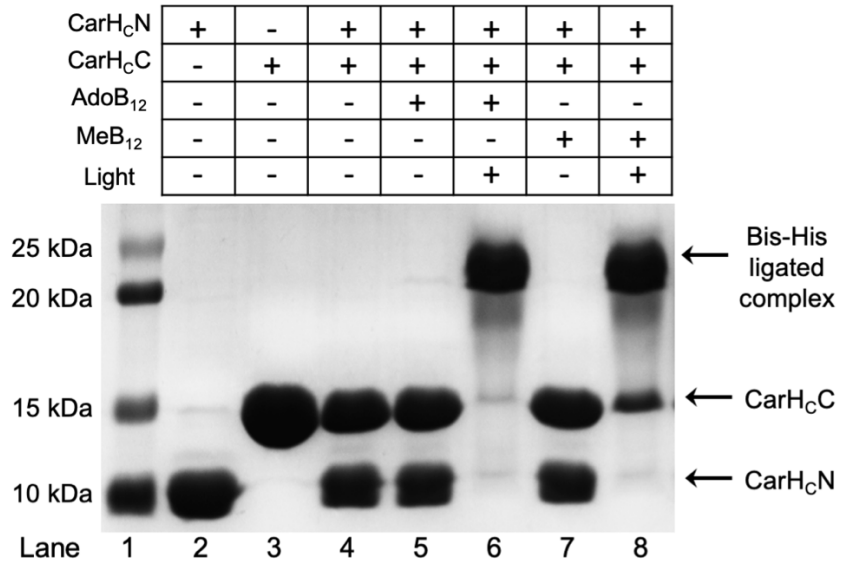


Fig. S6. SDS-PAGE analysis of the split-CarH$_C$ reconstitution induced by AdoB$_{12}$ and MeB$_{12}$. Protein samples were denatured using the 4% SDS loading dye (2X) at room temperature before the electrophoresis.

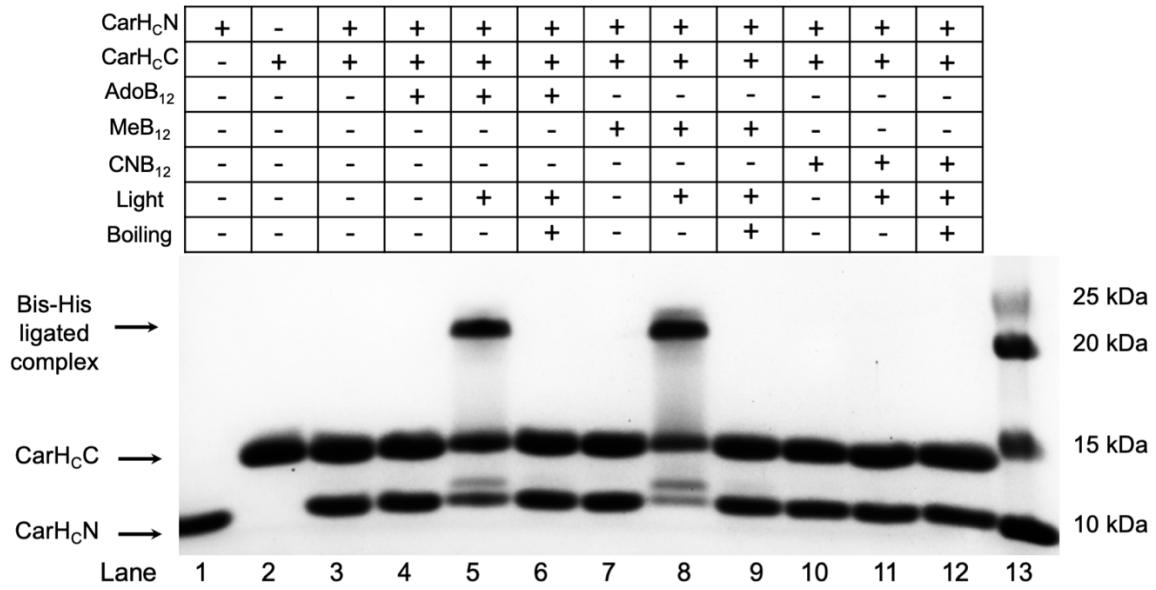


Fig. S7. SDS-PAGE analysis of the split-CarH$_C$ reconstitution induced by AdoB$_{12}$, MeB$_{12}$ and CNB$_{12}$. Protein samples were denatured with 2% SDS at room temperature or 100°C for 10 min before the electrophoresis.

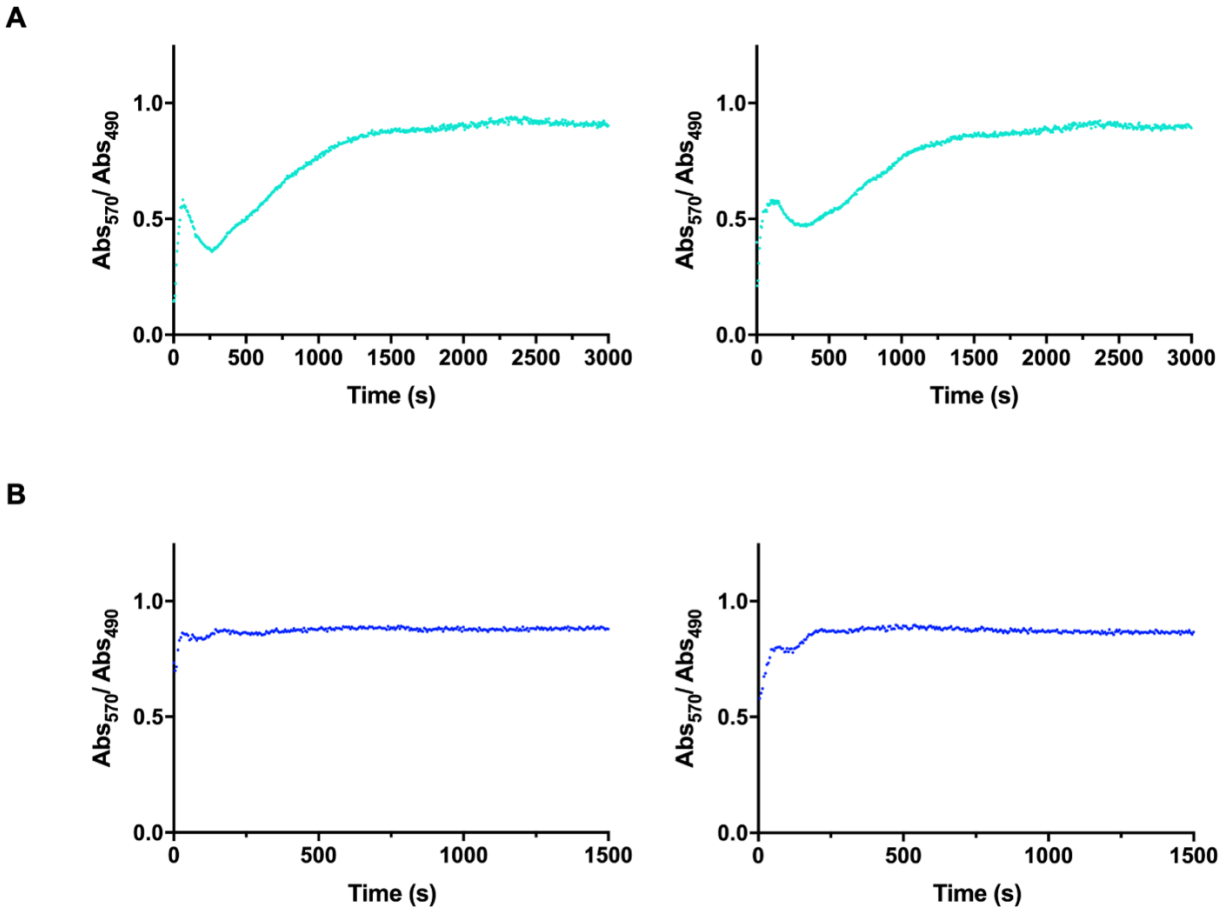


Fig. S8. Representative curves showing the binding kinetics of $AdoB_{12}$ + split $CarH_C$ (A) and $AdoB_{12}$ + $CarH_C$ (B) from two independent measurements. The concentrations of $AdoB_{12}$, split $CarH_C$ and $CarH_C$ were all 50 μM. Evolution of the ratio of the absorbance at 570 nm to that at 490 nm was recorded as a function of time.

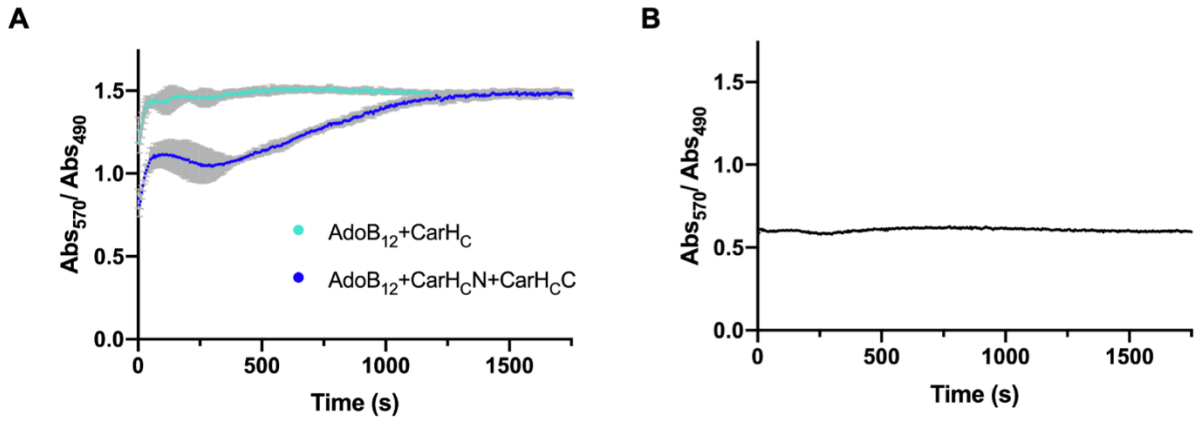


Fig. S9. Binding kinetics. (A) Binding kinetics of $AdoB_{12}$ (50 μM) to split $CarH_C$ or intact $CarH_C$ (50 μM). Evolution of the ratio of the absorbance at 570 nm to that at 490 nm was recorded as a function of time. Data are presented as mean ± SD (n = 5), with error bars in gray. (B) The corresponding Abs_{570}/ Abs_{490} of free $AdoB_{12}$ solution with the passage of time.

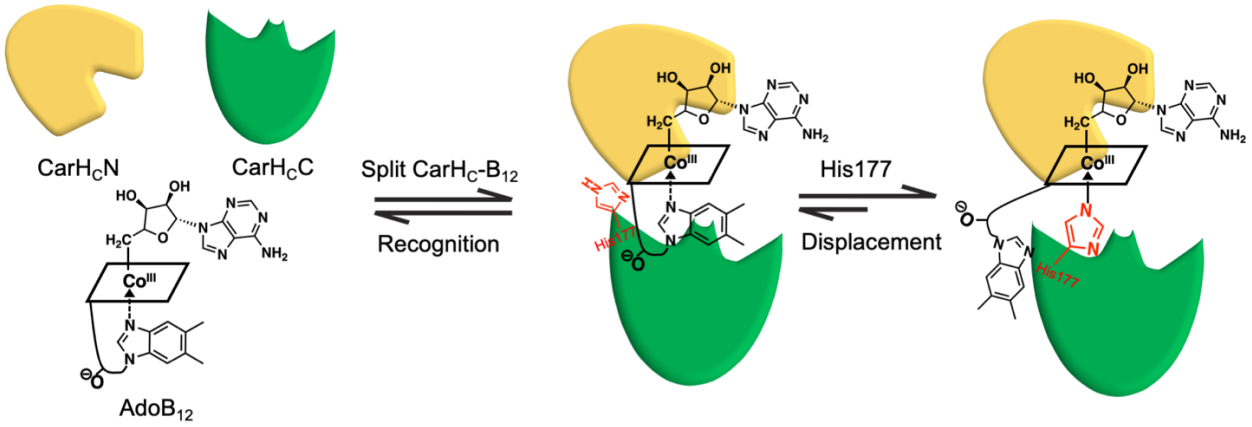


Fig. S10. Schematic illustration of the proposed binding mechanism of split $CarH_C$ with $AdoB_{12}$. The key residue His177 is highlighted in red.

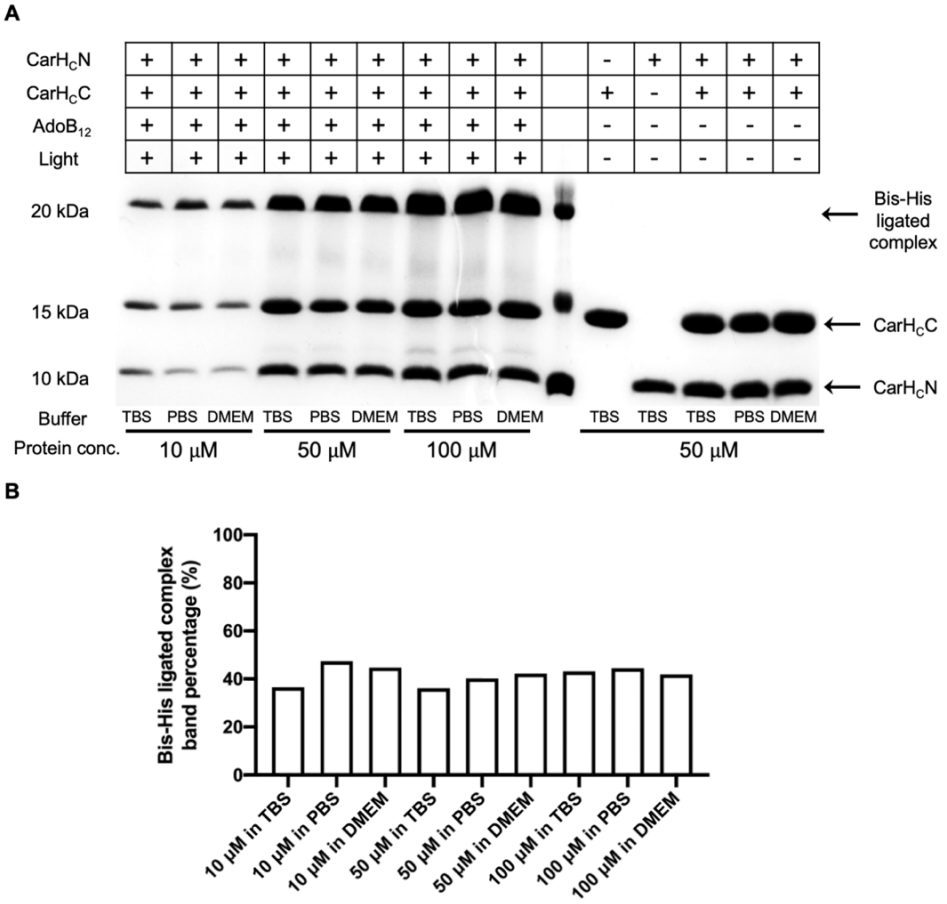


Fig. S11. SDS-PAGE analysis of the assembly of CarH$_C$N and CarH$_C$C with AdoB$_{12}$ under various conditions. The proteins, CarH$_C$N and CarH$_C$C, at an equimolar ratio were incubated with 2 *equiv.* AdoB$_{12}$ briefly in the dark for 20 min, followed by brief light exposure for 10 min, and then stopped by 4% SDS loading dye (2X) at room temperature. Shortened reaction time provided a snapshot for gauging the influence of different conditions on the assembly percentage. (A). According to the SDS-PAGE analysis below (B), the reactions, despite various buffer conditions and protein concentrations, all exhibited a similar yield (~40%) of the bis-His ligated product, which was quantified using the Image Lab software (Bio-Rad Laboratories). This result shows that this split CarH$_C$-based GECC is robust under varied buffer conditions (TBS, PBS and DMEM) and protein concentrations (10 to 100 μM).

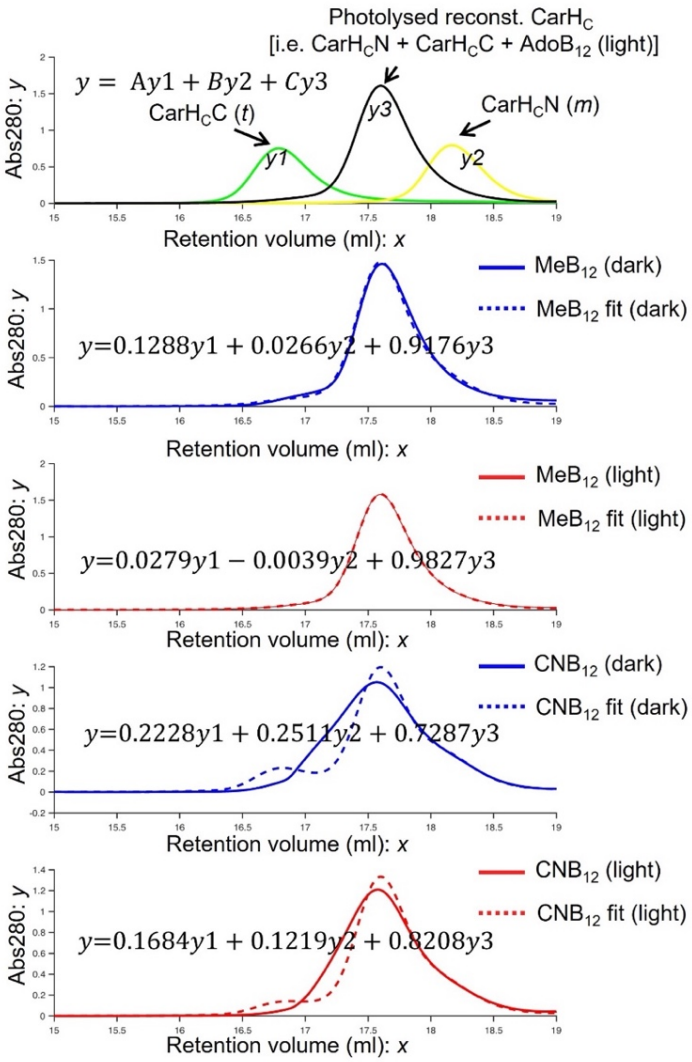


Fig. S12. Reconstitution efficiency with different cofactors. The results were deduced from the SEC data using MATLAB linear regression (the MATLAB code is provided as an appendix). Any observed SEC trace (y), which is a function of retention time (x), can be viewed as the linear combination of three types of protein molecules including $CarH_CN$, $CarH_CC$ and reconstituted $CarH_C$ (i.e., $CarH_CN$/$CarH_CC$ complex) and thus in principle can be deconvoluted into the corresponding three peaks, $y1$, $y2$ and $y3$. In the equation $y = Ay1 + By2 + Cy3$, A, B and C represent the relative percentages of these three molecules; C can therefore serve to measure reconstitution efficiency. To simplify this analysis, we used the $AdoB_{12}$-induced reconstitution as a reference and assumed that its reconstitution efficiency was 100% (i.e., C = 1). Stated in these terms, the efficiency of MeB_{12}-induced reconstitution is between ~92% (dark) and ~98% (light), respectively, while this number of CNB_{12} stood between ~73% and ~82%, suggesting a relatively lower reconstitution efficiency with CNB_{12}.

ACCA (33.4 kDa)
SpyTag-ELP-CarH$_C$C-ELP-SpyTag

MKGSSHHHHHHVDAHIVMVDAYKPTKLDGHGVGVPGVGVPGVGVPGEGVPGVGV
PGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGV
GELGRGDGGGGSGGGGSPPGPPVLVTTPPGERHEIGAMLAAYHLRRKGVPALYLG
PDTPLPDLRALARRLGAGAVVLSAVLSEPLRALPDGALKDLAPRVFLGGQGAGPEEA
RRLGAEYMEDLKGLAEALWLPRGPEKEAITSVPGVGVPGVGVPGEGVPGVGVPGV
GVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVP
GGLLDAHIVMVDAYKPTKLEWKK

BCNB (56.0 kDa)
SpyCatcher-ELP-CarH$_C$N-ELP-SpyCatcher

MKGSSHHHHHHVDIPTTENLYFQGAMVDTLSGLSSEQGQSGDMTIEEDSATHIKFSK
RDEDGKELAGATMELRDSSGKTISTWISDGQVKDFYLYPGKYTFVETAAPDGYEVAT
AITFTVNEQGQVTVNGKATKGDAHIDGPQGIWGQLEGHGVGVPGVGVPGVGVPGE
GVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVP
GVGVPGVGELGRGDGGGGSGGGGSPEDLGTGLLEALLRGDLAGAEALFRRGLRF
WGPEGVLEHLLLPVLREVGEAWHRGEIGVAEEHLASTFLRARLQELLDLAGFPPGPP
TSVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVP
GVGVPGVGVPGEGVPGVGVPGVGVPGGLVDIPTTENLYFQGAMVDTLSGLSSEQG
QSGDMTIEEDSATHIKFSKRDEDGKELAGATMELRDSSGKTISTWISDGQVKDFYLYP
GKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGKATKGDAHIDGPQGIWGQLEWKK

ACNA (29.8 kDa)
SpyTag-ELP-CarH$_C$C-ELP-SpyTag

MKGSSHHHHHHVDAHIVMVDAYKPTKLDGHGVGVPGVGVPGVGVPGEGVPGVGV
PGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGV
GELGRGDGGGGSGGGGSPEDLGTGLLEALLRGDLAGAEALFRRGLRFWGPEGVLE
HLLLPVLREVGEAWHRGEIGVAEEHLASTFLRARLQELLDLAGFPPGPPTSVPGVGV
PGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGV
GVPGEGVPGVGVPGVGVPGGLLDAHIVMVDAYKPTKLEWKK

BCCB (59.5 kDa)
SpyCatcher-ELP-CarH$_C$N-ELP-SpyCatcher

MKGSSHHHHHHVDIPTTENLYFQGAMVDTLSGLSSEQGQSGDMTIEEDSATHIKFSK
RDEDGKELAGATMELRDSSGKTISTWISDGQVKDFYLYPGKYTFVETAAPDGYEVAT
AITFTVNEQGQVTVNGKATKGDAHIDGPQGIWGQLEGHGVGVPGVGVPGVGVPGE
GVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVP
GVGVPGVGELGRGDGGGGSGGGGSPPGPPVLVTTPPGERHEIGAMLAAYHLRRK
GVPALYLGPDTPLPDLRALARRLGAGAVVLSAVLSEPLRALPDGALKDLAPRVFLGG
QGAGPEEARRLGAEYMEDLKGLAEALWLPRGPEKEAITSVPGVGVPGVGVPGEGV
PGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGV
GVPGVGVPGGLVDIPTTENLYFQGAMVDTLSGLSSEQGQSGDMTIEEDSATHIKFSK
RDEDGKELAGATMELRDSSGKTISTWISDGQVKDFYLYPGKYTFVETAAPDGYEVAT
AITFTVNEQGQVTVNGKATKGDAHIDGPQGIWGQLEWKK

Fig. S13. Amino acid sequences of SpyTag-ELP-CarH$_C$C-ELP-SpyTag (ACCA), SpyCatcher-ELP-CarH$_C$N-ELP-SpyCatcher (BCNB), SpyTag-ELP-CarH$_C$N-ELP-SpyTag (ACNA) and SpyCatcher-ELP-CarH$_C$C-ELP-SpyCatcher (BCCB). Sequences of SpyTag, SpyCatcher, CarH$_C$N and CarH$_C$C are highlighted in red, purple, yellow, and green, respectively. Sequences of RGD cell binding motifs and MMP sites are underlined and boxed, respectively.

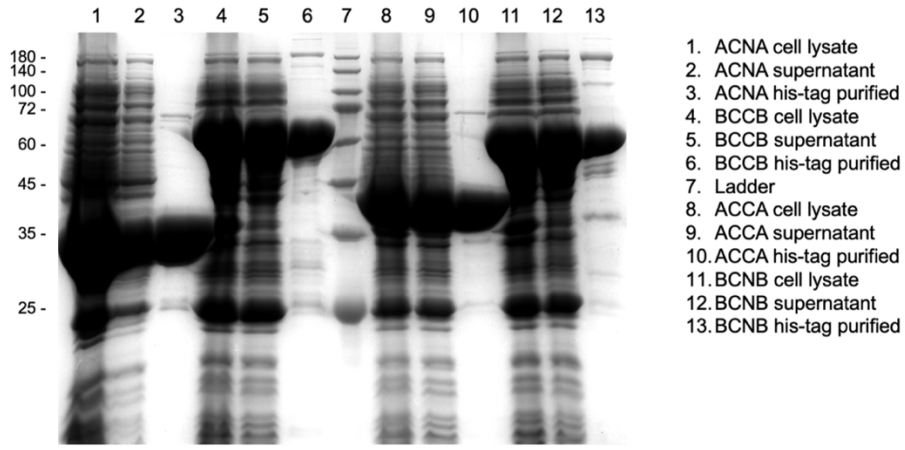


Fig. S14. SDS-PAGE analysis of ACNA (29.8 kDa), BCCB (59.5 kDa), ACCA (33.4 kDa) and BCNB (56.0 kDa).

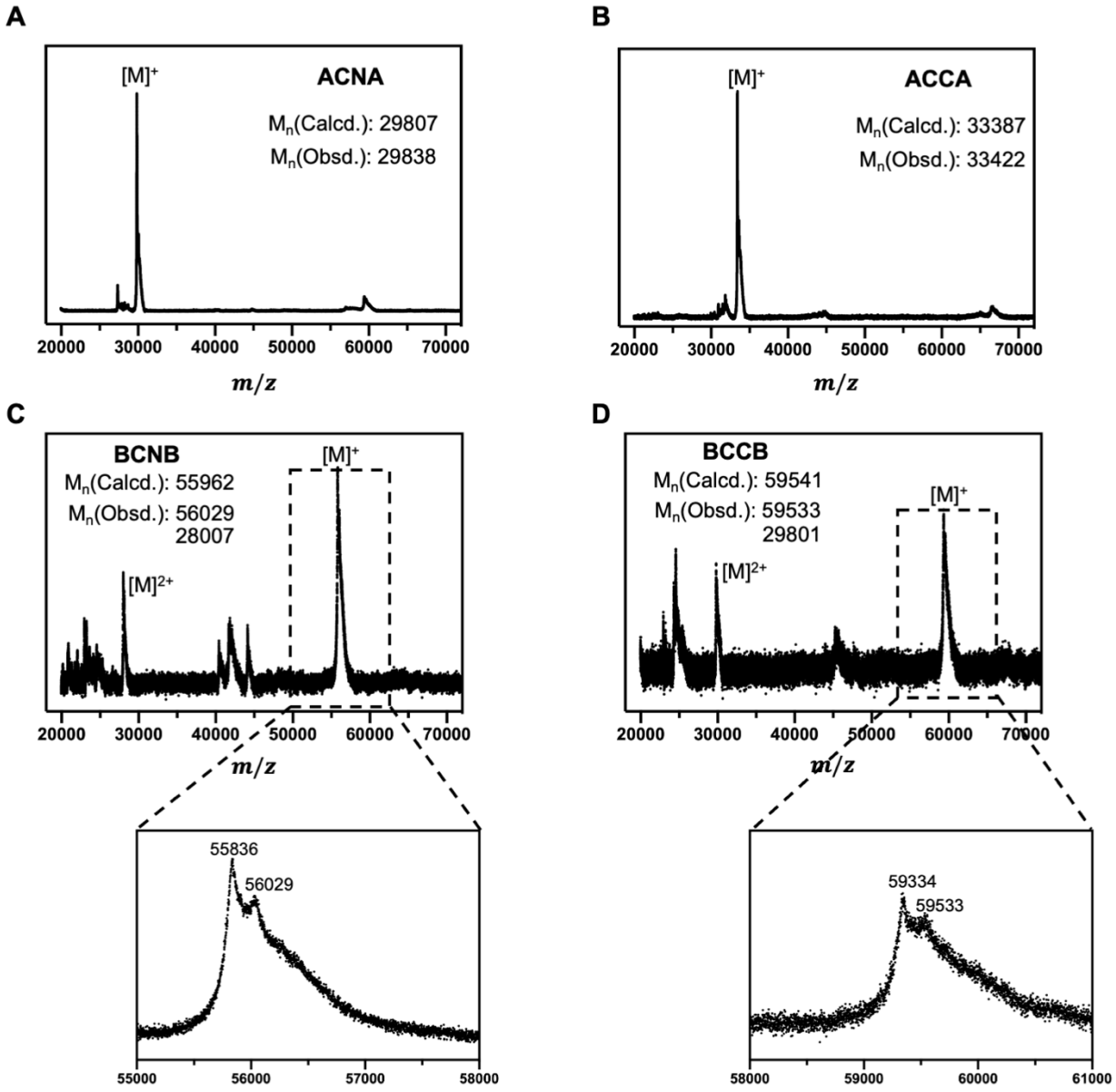


Fig. S15. MALDI-TOF mass spectra of (A) ACNA, (B) ACCA, (C) BCNB and (D) BCCB.

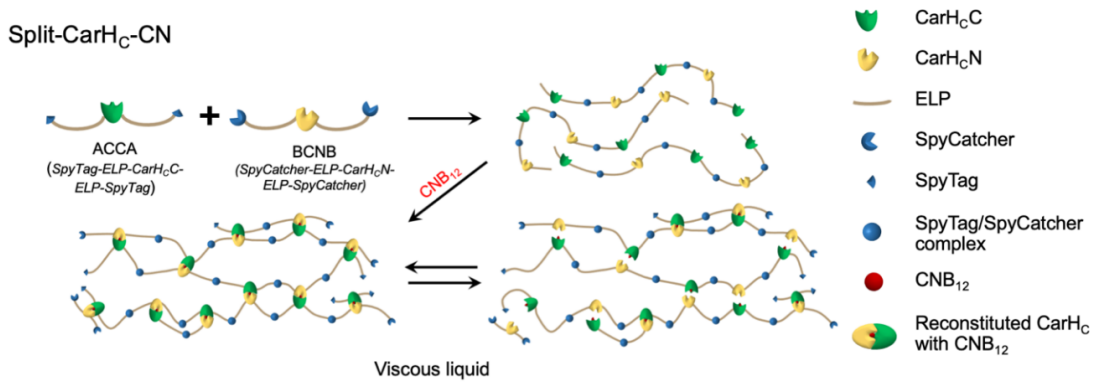


Fig. S16. Schematic showing the formation of a physical network comprising ACCA + BCNB + CNB_{12}.

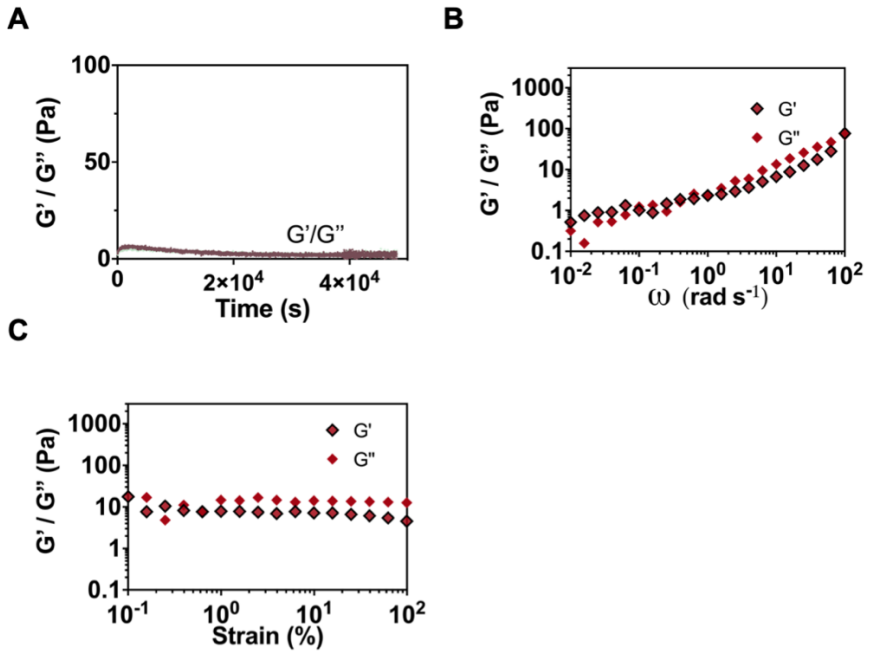


Fig. S17. Rheological characterization showing that the product of ACCA+ BCNB at an equimolar ratio in the absence of B_{12} is a liquid. (A) Evolution of the storage modulus G' and loss modulus G" of ACCA + BCNB free of B_{12} at 23 °C as a function of time with a fixed frequency of 1 rad/s and strain of 5% at 23 °C. (B) Frequency-sweep test at 23 °C with the strain fixed at 5%. (C) Strain-sweep test at 23 °C with the frequency fixed at 1 rad/s.

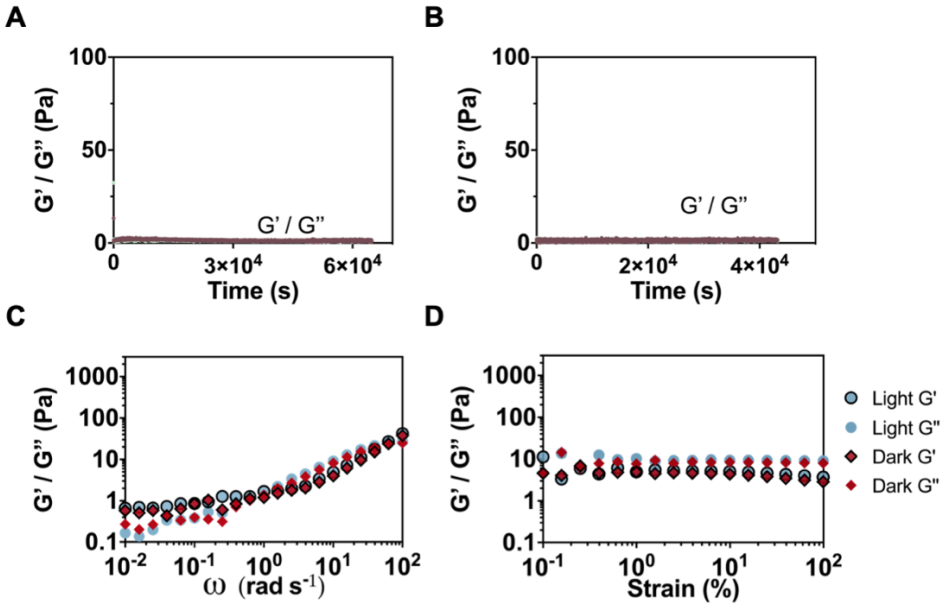


Fig. S18. Rheological characterization showing that the product of ACCA+BCNB+CNB_{12} remains as a liquid before and after light exposure. (A and B) Evolution of the storage modulus G' and loss modulus G" of ACCA+BCNB+CNB_{12} at 23 °C in the dark (A) and under light illumination (B) as a function of time. The time-sweep tests were performed at a fixed frequency of 1 rad/s and strain of 5% at 23 °C. (C) Frequency-sweep tests at a fixed strain of 10% at 23 °C. (D) Strain-sweep tests at a fixed frequency of 10 rad/s at 23 °C.

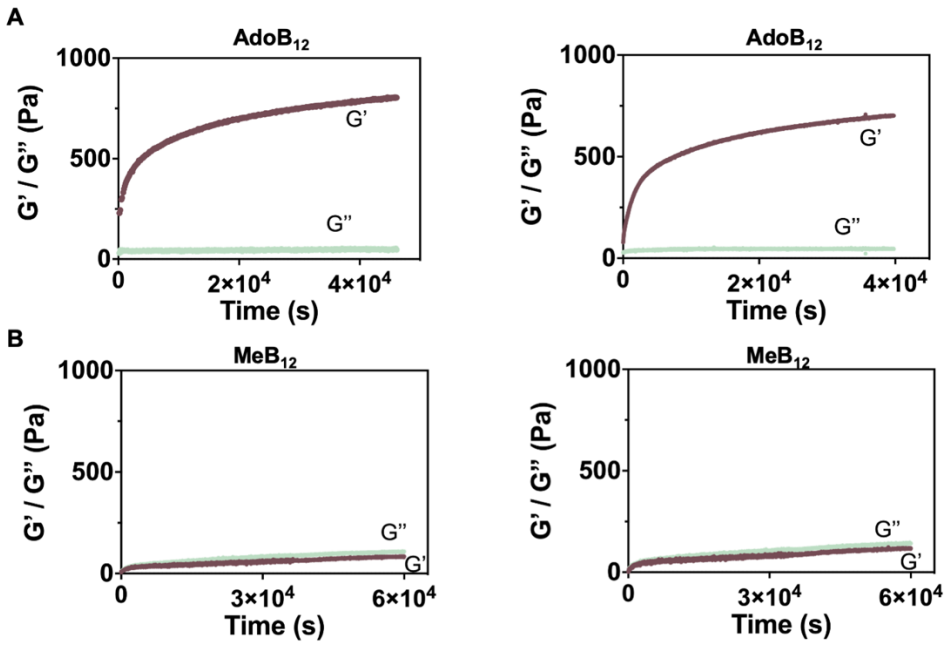


Fig. S19. Two additional replicates of time-sweep tests of products of (A) ACCA + BCNB + $AdoB_{12}$ and (B) ACCA + BCNB + MeB_{12} in the dark. Experiments were performed at a fixed frequency of 1 rad/s and strain of 5% at 23 °C.

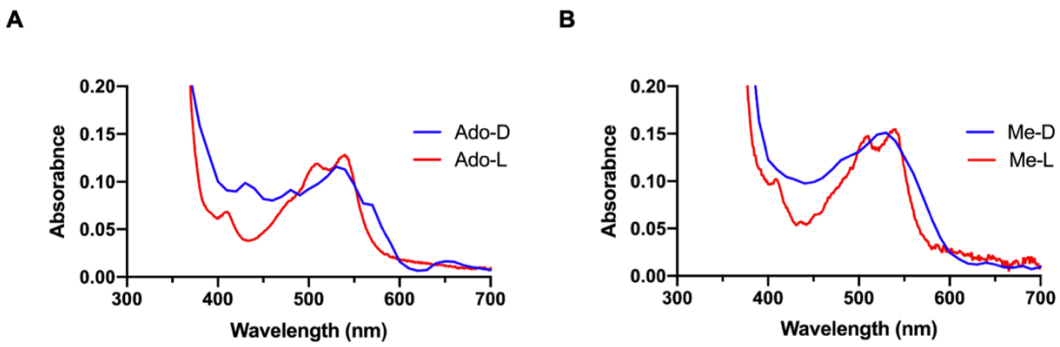


Fig. S20. UV-vis spectra of Ado-D, Ado-L (A), Me-D and Me-L (B) hydrogels.

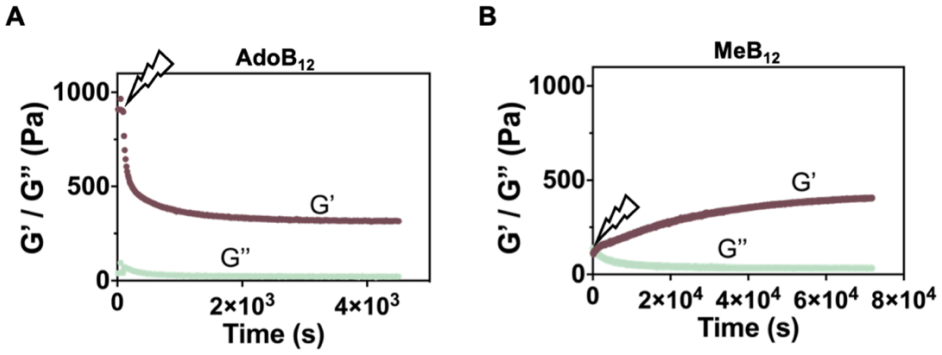


Fig. S21. Photo-weakening of Ado gel (A) and photo-strengthening of Me gel (B) monitored by time-sweep tests at a fixed frequency of 1 rad/s and strain of 5% at 23 °C.

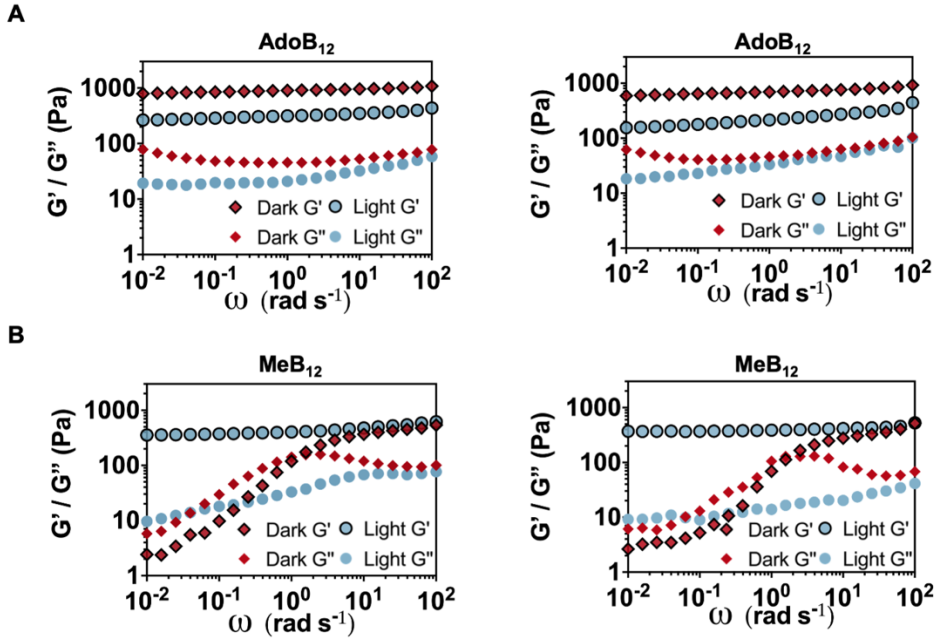


Fig. S22. Two additional replicates of frequency-sweep tests of the products of (A) ACCA + BCNB + $AdoB_{12}$ and (B) ACCA + BCNB + MeB_{12} before and after photolysis. Experiments were performed at a fixed strain of 10% at 23 °C.

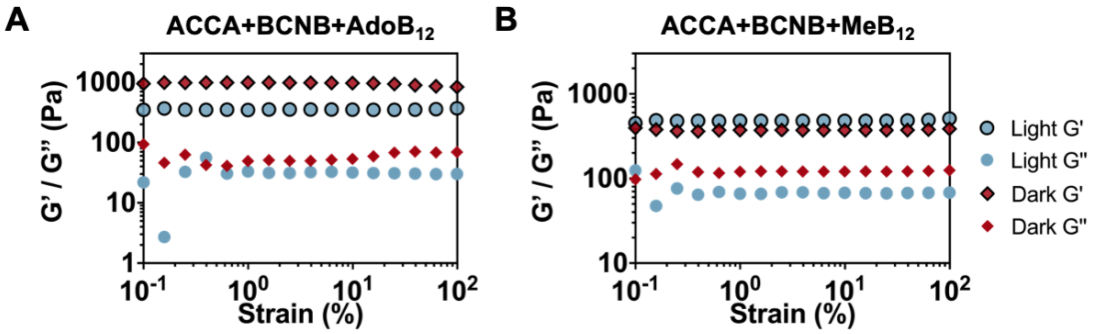


Fig. S23. Strain-sweep tests of the products of ACCA + BCNB + $AdoB_{12}$ (A) and ACCA + BCNB + MeB_{12} (B) before and after photolysis. Experiments were performed at a fixed frequency of 10 rad/s at 23 °C.

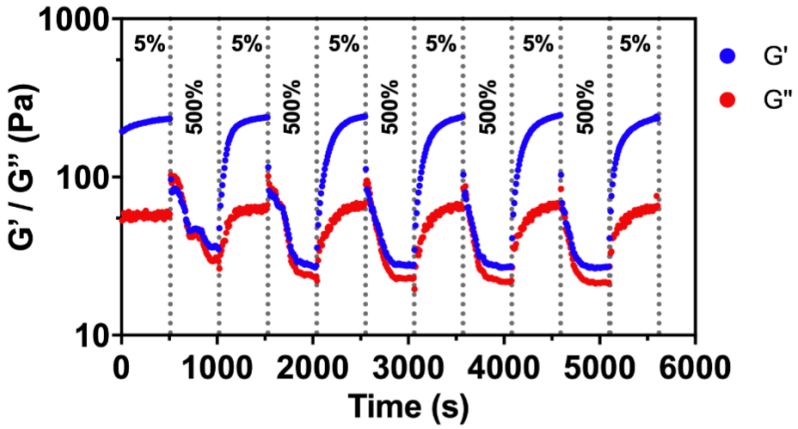


Fig. S24. Continuous step-strain test of Me-D. Low (5%) and high strains (500%) were used alternately. Experiments were performed at a fixed frequency of 10 rad/s at 23 °C.

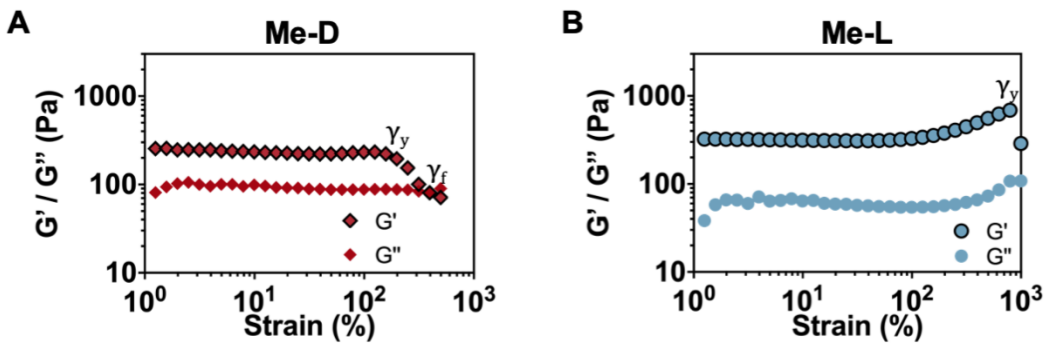


Fig. S25. Large amplitude strain-sweep tests of the products of ACCA + BCNB + MeB_{12} before (A) and after (B) photolysis. The results show that photo-strengthening of the gels increases their strain linearity limit. Experiments were performed at a fixed frequency of 10 rad/s at 23 °C.

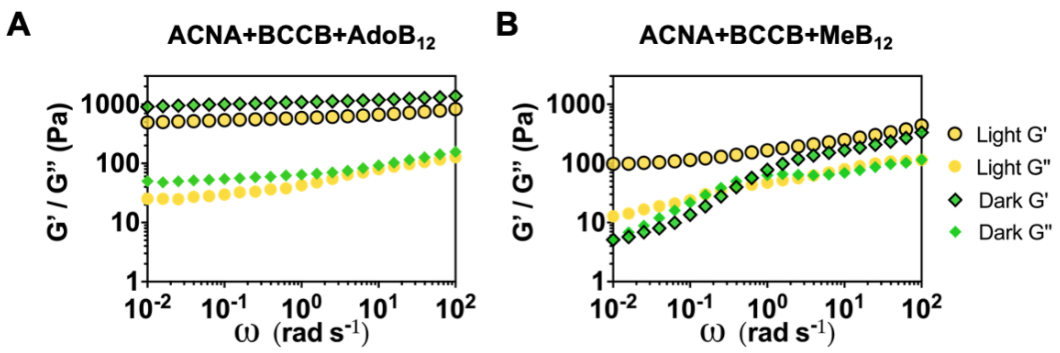


Fig. S26. Frequency-sweep tests of the products of ACNA + BCCB + $AdoB_{12}$ (A) and ACNA + BCCB + MeB_{12} (B) before and after photolysis. Experiments were performed at a fixed strain of 10% at 23 °C.

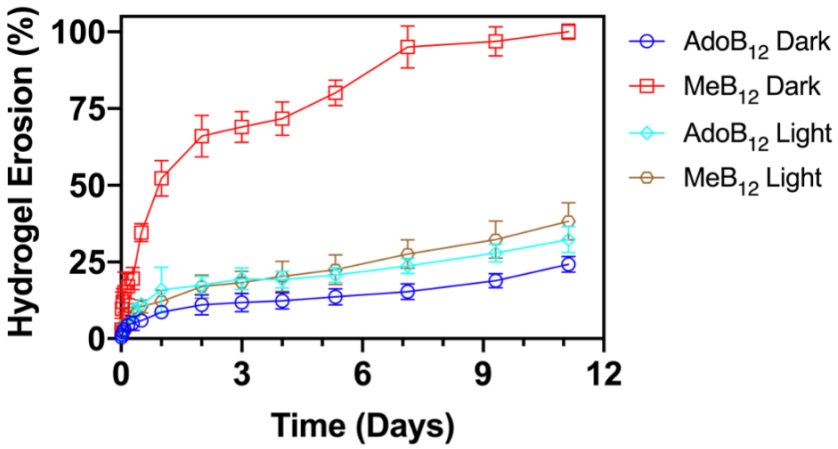


Fig. S27. Erosion profiles of Ado and Me gels. Data are presented as mean ± SD (n = 5). Hydrogels were prepared by mixing 16.6 μL of ACCA (10 wt % in PBS), 27.8 μL of BCNB (10 wt % in PBS) and 5.4 μL of $AdoB_{12}$ or MeB_{12} (10 mM in PBS). The products were cured in the dark for 24 h. Half of the resulting materials were further exposed to 30 kilolux light for another 24 h. Then all samples were immersed by 1 mL of PBS (pH 7.4). Aliquots of 75 μL were taken at defined time points and fresh PBS (75 μL) was added back to keep the volume constant. All the experiments were performed at 23 °C. The absorbances of these aliquots at 280 and 522 nm were measured using a NANODROP 2000C spectrophotometer (Thermo Scientific). Protein concentrations were calculated based on the previous published equations (24):

$$A_{280}(protein) = A_{280} - A_{522} * \frac{A_{280}(AdoB_{12}, 100\mu M)}{A_{522}(AdoB_{12}, 100\mu M)}$$

$$C(protein) = \frac{A_{280}(protein)}{Ext.coeff}$$

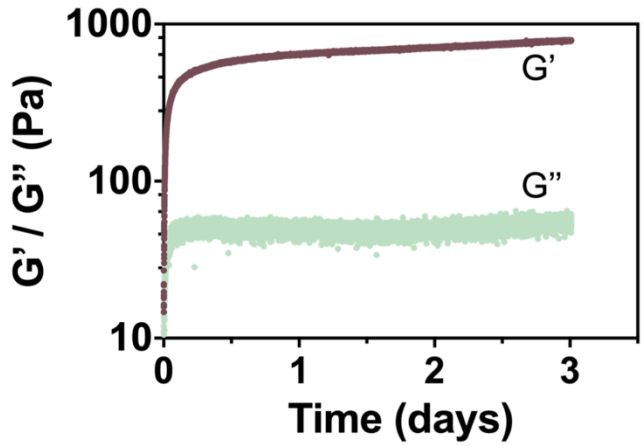


Fig. S28. Time-sweep test of ACCA + BCNB + AdoB$_{12}$ in the dark. Experiments was performed at a fixed frequency of 1 rad/s and strain of 5% at 23 °C.

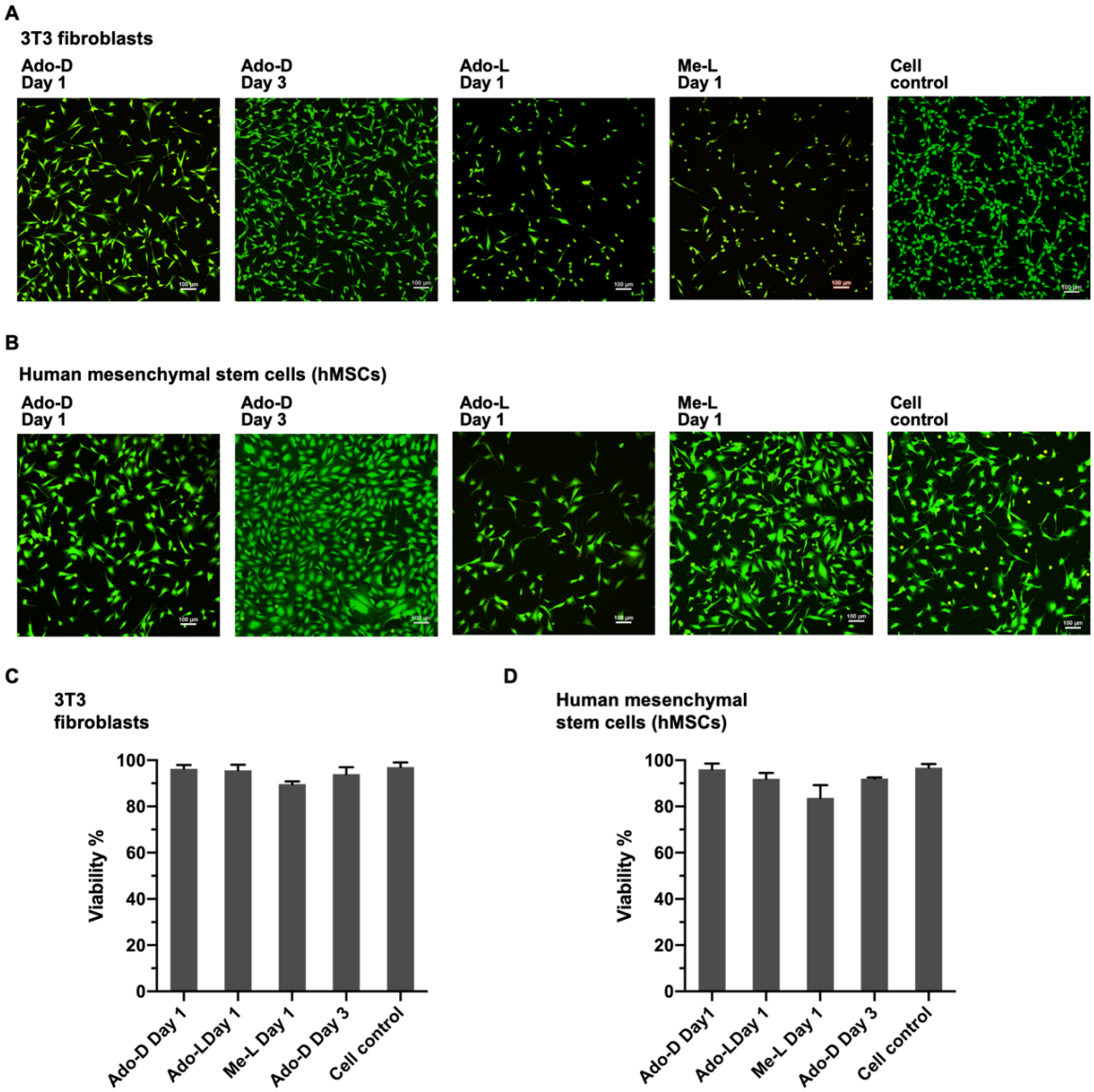


Fig. S29. Influence of gel precursors on cell viability. (A and B) Representative images of 3T3 fibroblasts (A) and human mesenchymal stem cells (hMSCs) (B) cultured in growth medium (1 mL) spiked with gel precursors including ACCA (1.7 mg or 50 nmol), BCNB (2.8 mg or 50 nmol), and $AdoB_{12}$ or MeB_{12} (56 nmol). The dry weights of these gel precursors in 1 mL of growth medium were the same as in the cell encapsulation experiments. Three independent experiments were performed. Cell cultures free of gel precursors were used as control groups. (C and D) Cell viability was calculated as the ratio of live cell population to entire cell population and presented as mean ± SD (n = 3).

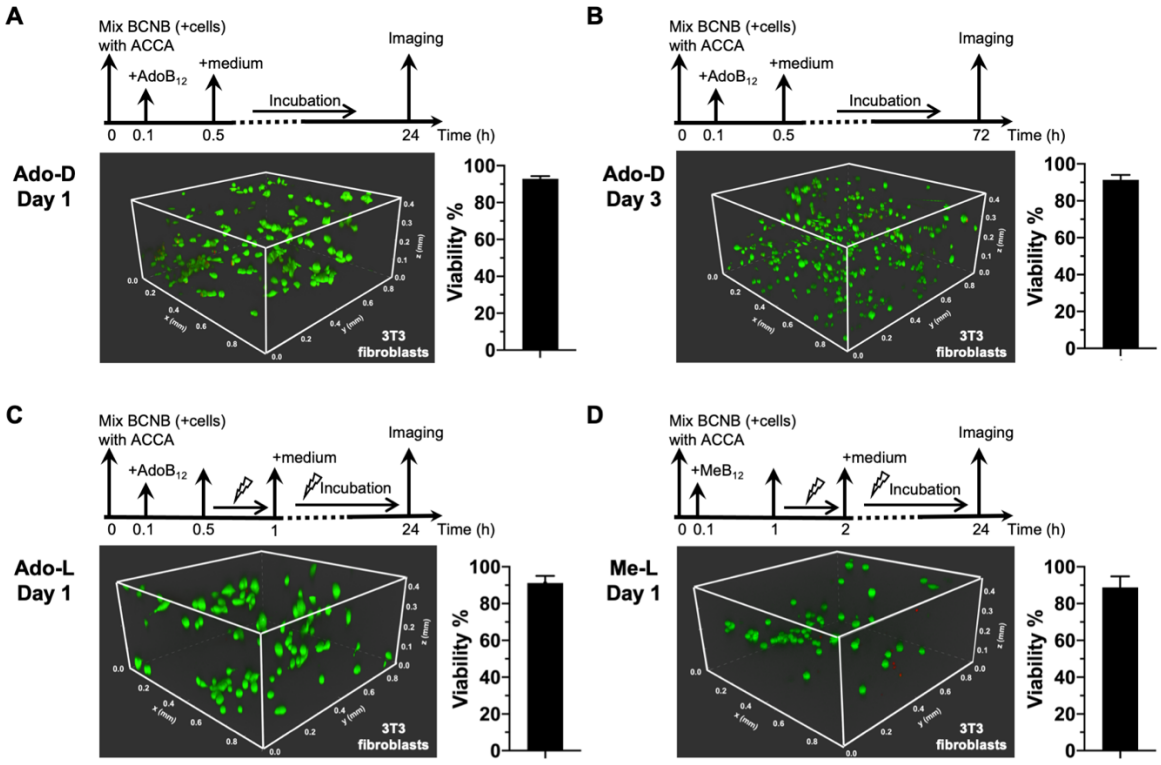


Fig. S30. Encapsulation of mouse 3T3 fibroblasts. (A) One-day culturing with an Ado gel in the dark. (B) Three-day culturing with an Ado gel in the dark. (C) One-day culturing with a photo-weakened Ado gel. (D) One-day culturing with a photo-strengthened Me gel. Cell viability was assessed by the standard live (green) /dead (red) staining assay. Three independent cell encapsulation experiments were performed, each time done in triplicate (with three identical gels prepared for each corresponding encapsulation condition). Cell viability under each condition was calculated as the ratio of live cell population to entire cell population based on the three parallel experiments and presented as mean ± SD (n = 3).

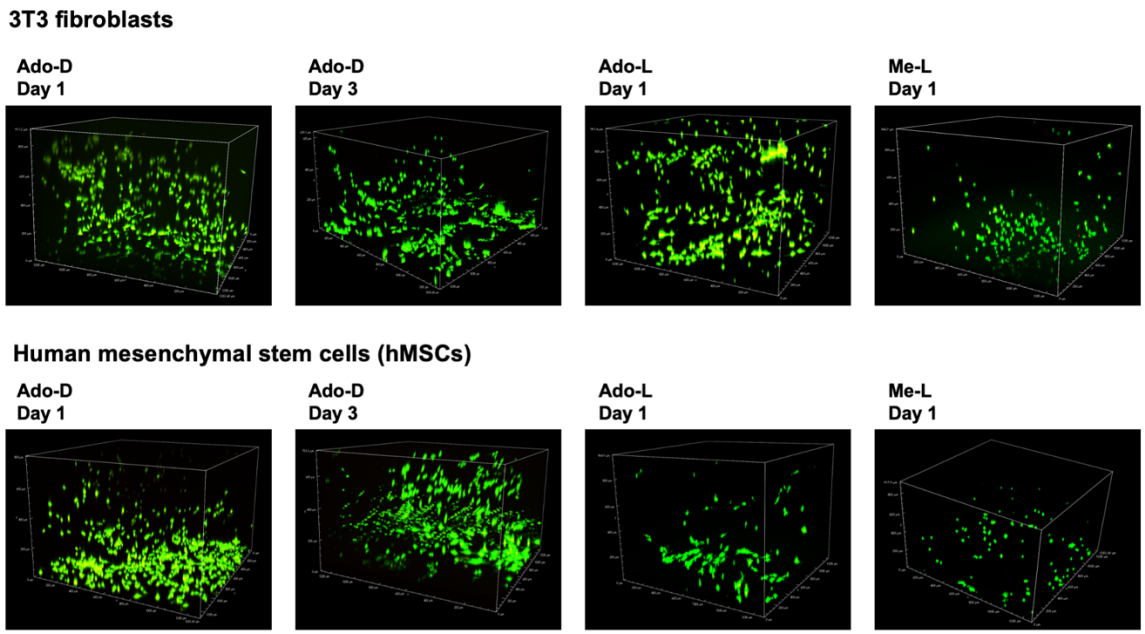


Fig. S31. Images of 3D encapsulation of 3T3 fibroblasts and hMSCs obtained from two additional independent experiments. Cell viability was assessed by the standard live (green) /dead (red) staining assay.

Table S1. Bacterial strains, plasmids, and primers used in this study.

Strain	**Relevant Characteristics**	**Source**
E.coli DH5α		Stratagene
E.coli BL21 star(DE3)		Invitrogen
Plasmid	**Relevant Characteristics**	**Source**
pET32m3C	T7 promoter-operator, His tag, Trx solubilization tag, HRV 3C site, Amp^r	Prof. Mingjie Zhang (46)
pET32m3C-*carH$_C$N*	Plasmid for expression of CarH$_C$N	This study
pET32m3C-*carH$_C$C*	Plasmid for expression of CarH$_C$C	This study
pQE-*AAA*	Plasmid for expression of SpyTag-ELP-SpyTag-ELP-SpyTag	Previously published construct (25)
pQE-*ACNA*	Plasmid for expression of SpyTag-ELP-CarH$_C$N-ELP-SpyTag	This study
pQE-*ACCA*	Plasmid for expression of SpyTag-ELP-CarH$_C$C-ELP-SpyTag	This study
pQE-*BB*	Plasmid for expression of SpyCatcher-ELP-RGD-ELP-SpyCatcher	Previously published construct (25)
pQE-*BCNB*	Plasmid for expression of SpyCatcher-CarH$_C$N-RGD-ELP-SpyCatcher	This study
pQE-*BCCB*	Plasmid for expression of SpyCatcher-CarH$_C$C-RGD-ELP-SpyCatcher	This study
Primer	**Relevant Characteristics**	**Source**
*carH$_C$N*_EcoRI_F	CCGGAATTCCCAGAAGATCTGGGCACCGG	Genewiz
*carH$_C$N*_HindIII_R	CCCAAGCTTTTACGGCGGACCCGGCGGGAAAC	Genewiz
*carH$_C$C*_EcoRI_F	CCGGAATTCCCGCCGGGTCCGCCGGTCCT	Genewiz
*carH$_C$C*_HindIII_R	CCCAAGCTTTTAGATTGCTTCTTTTTCCGGAC	Genewiz
*carH$_C$N*_SacI_F	GGTGAGCTCGGCCGTGGTGATGGTGGTGGGGGTAGCGGTGGTGGGGGTAGTCCAGAAGATCTGGGCACCGG	Genewiz
*carH$_C$N*_SpeI_R	CGGACTAGTCGGCGGACCCGGCGGGAAAC	Genewiz
*carH$_C$C*_SacI_F	GGTGAGCTCGGCCGTGGTGATGGTGGTGGGGGTAGCGGTGGTGGGGGTAGTCCGCCGGGTCCGCCGGTCCT	Genewiz
*carH$_C$C*_SpeI_R	CGGACTAGTGATTGCTTCTTTTTCCGGAC	Genewiz

Appendix:
###MATLAB code (size-exclusion chromatography data fitting)

```
load FeiData2.mat

% C: CarHcC(t) g
% N: CarHcN(t) y
% NC: k
% MeDark: b
% MeLight: r

filter = x>15 & x<=22;
x=x(filter);
C=C(filter);
N=N(filter);
NC=NC(filter);
MeDark=MeDark(filter);
MeLight=MeLight(filter);
CNDark=CNDark(filter);
CNLight=CNLight(filter);

figure;
subplot(5,1,1)
plot(x,C,'g','linewidth',4)
hold on
plot(x,N,'y','linewidth',4)
plot(x,NC,'k','linewidth',4)
set(gca,'tickdir','out','linewidth',2,'fontsize',16)
legend({'CarH_CN','CarH_CC','CarH_CN+CarH_CC'})

subplot(5,1,2)
plot(x,MeDark,'b','linewidth',4)
hold on

b1=[N C NC]\MeDark;

plot(x,b1(1)*N+b1(2)*C+b1(3)*NC,'b--','linewidth',4)
set(gca,'tickdir','out','linewidth',2,'fontsize',16)
legend({'MeDark','MeDark-Fit'})


subplot(5,1,3)
plot(x,MeLight,'r','linewidth',4)
hold on

b2=[N C NC]\MeLight;

plot(x,b2(1)*N+b2(2)*C+b2(3)*NC,'r--','linewidth',4)
set(gca,'tickdir','out','linewidth',2,'fontsize',16)
legend({'MeLight','MeLight-Fit'})


subplot(5,1,4)
plot(x,CNDark,'b','linewidth',4)
hold on

b3=[N C NC]\CNDark;
```

```
plot(x,b3(1)*N+b3(2)*C+b3(3)*NC,'b--','linewidth',4)
set(gca,'tickdir','out','linewidth',2,'fontsize',16)
legend({'CNDark','CNDark-Fit'})


subplot(5,1,5)
plot(x,CNLight,'r','linewidth',4)
hold on

b4=[N C NC]\CNLight;

plot(x,b4(1)*N+b4(2)*C+b4(3)*NC,'r--','linewidth',4)
set(gca,'tickdir','out','linewidth',2,'fontsize',16)
legend({'CNLight','CNLight-Fit'})
```