Supporting Information

Hanson *et al.* 10.1073/pnas.0810318105

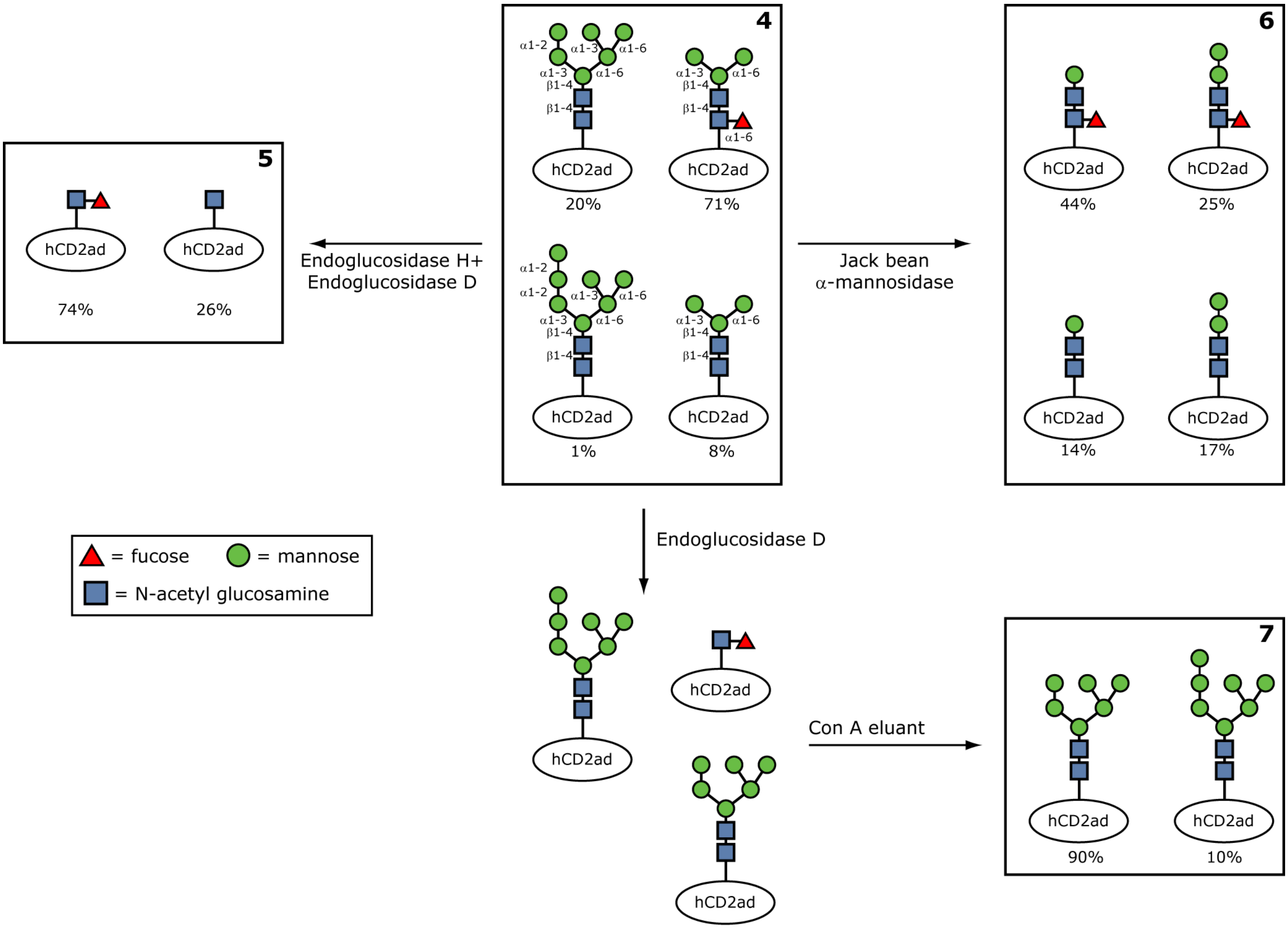


Fig. S1. Enzymatic remodeling of hCD2ad. Numbers under glycoforms indicate the percent of each glycoform in the mixture.

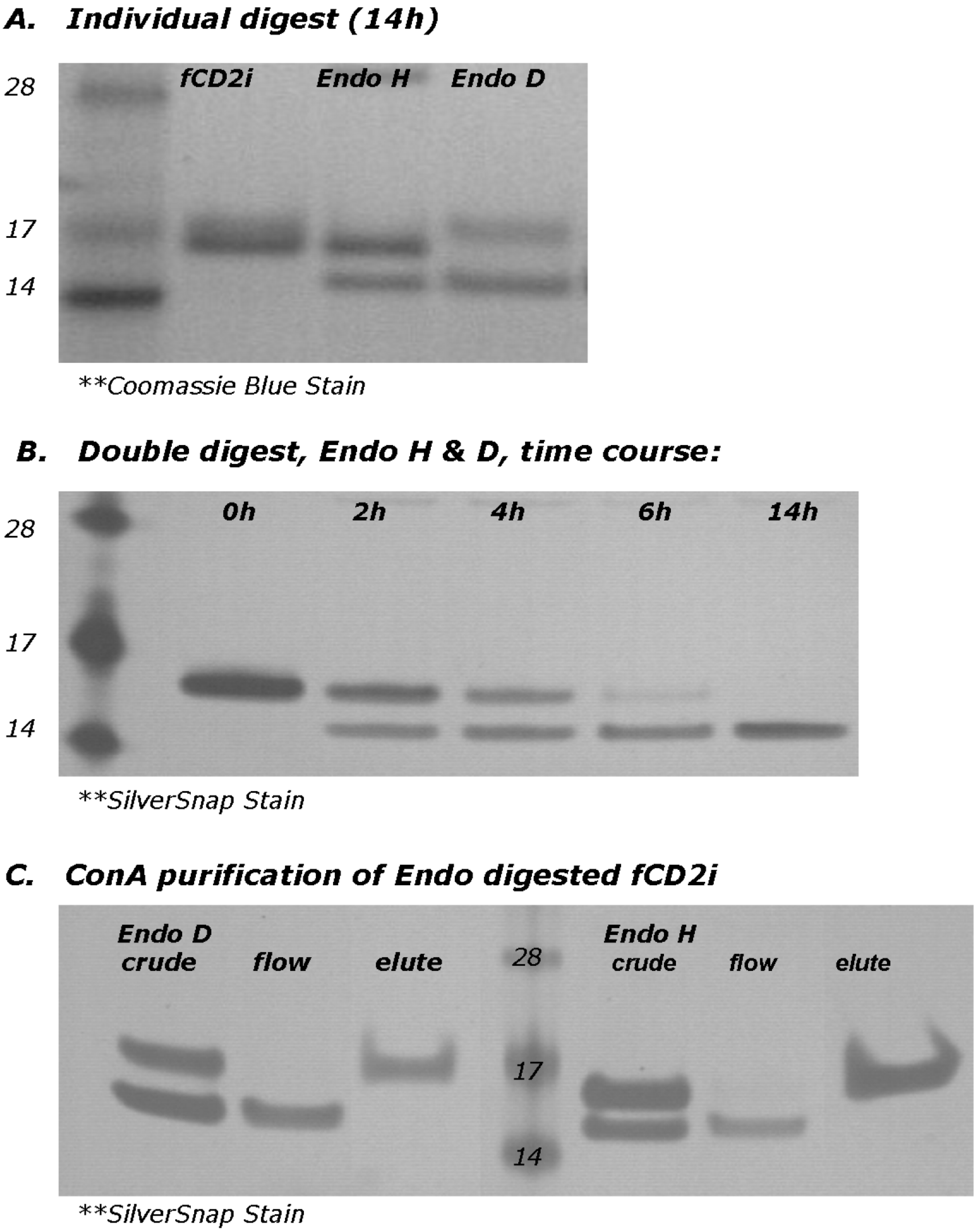


Fig. S2. Analysis of enzymatic remodeling of hCD2ad variant 4 (labeled as fCD2i in the figure) with endoglycosidases by SDS/PAGE.

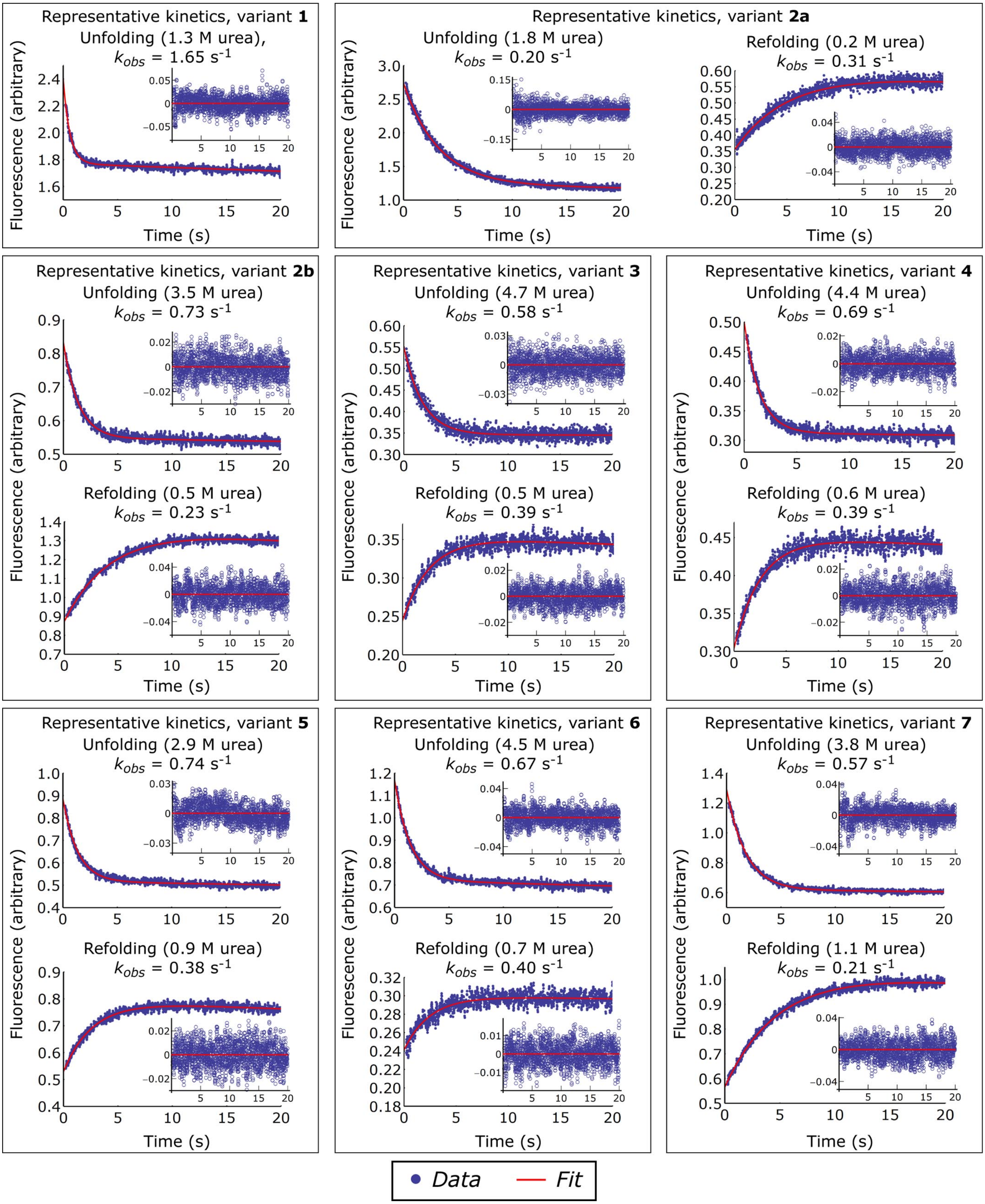
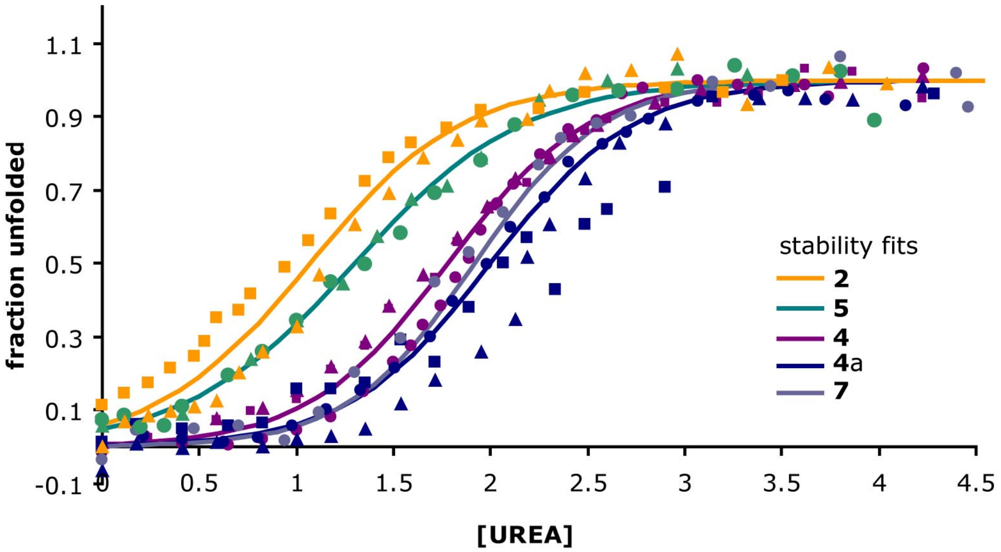


Fig. S3. Representative hCD2ad variant kinetic plots and fits. Residuals are shown as insets.



hCD2ad variant		Stability from Thermodynamics $\Delta G_{f,0}$ (kcal/mol)	m_{eq} (kcal/mol M)	n	Stability from Kinetics $\Delta G_{f,0}$ (kcal/mol)	m_{eq} (kcal/mol M)
2	●	-1.6±0.4	1.5±0.2	2	-1.5±0.1	1.62±0.09
5	●	-1.8±0.3	1.4±0.1	2	-1.6±0.1	1.53±0.14
4	●	-2.9±0.2	1.6±0.1	3	-2.9±0.2	1.57±0.14
4a	●	-3.2±0.8	1.5±0.2	3	—	—
7	●	-3.5±0.8	1.8±0.3	2	-2.5±0.1	1.66±0.08

Fig. S4. Representative hCD2ad variant urea denaturation curves. Stabilities obtained by equilibrium thermodynamics and kinetics are in agreement, which is a good indication of 2-state behavior.

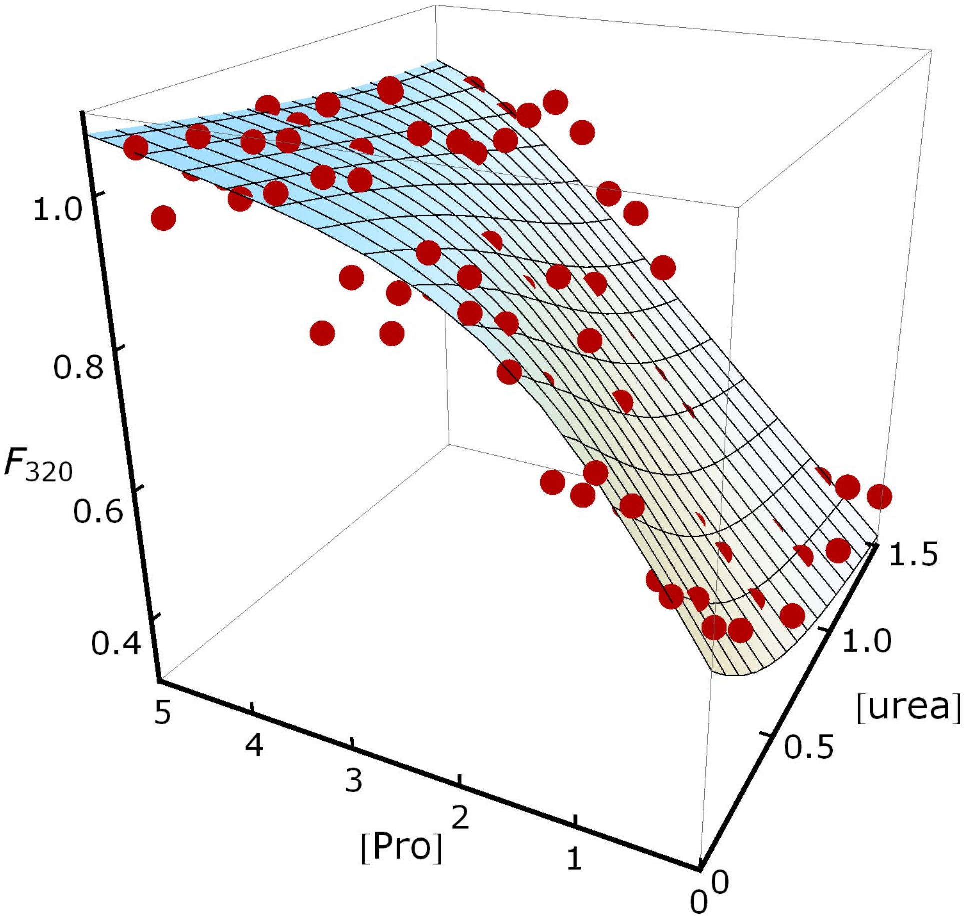


Fig. S5. hCD2ad variant **1** urea/proline denaturation/renaturation surface and fit. Points represent experimental F_{320} values and the surface represents the fit to these values. F_{320} is measured in arbitrary units.

Table S1. MS characterization of hCD2ad glycoforms from HEK cells (hCD2ad variant 3)

N-glycan type	MS_{found}	RA	Structure
Hybrid	15,441	100	$GlcNAcMan_5GlcNAc_2$
Complex	15,646	79	$LacNAc_2ManGlcNAc_2$
Oligomannose	15,372	67	$Man_6GlcNAc_2$
Complex	15,793	60	$LacNAc_2ManGlcNAc_2 + Fuc$
Hybrid	15,591	60	$LacNAc_2Man_5GlcNAc_2$
Hybrid	15,282	56	$LacNAc_2Man_4GlcNAc_2$

RA, relative abundance

Table S2. MS characterization of hCD2ad insect glycoforms

		RA			
N-glycan type	$MW_{expected}$	Sf9-1	Sf9-2	Sf9-3	Tn5
$Man_3GlcNAc_2(Fuc)$	15,031	74	70	70	55
$Man_6GlcNAc_2$	15,373	15	24	20	28
$Man_3GlcNAc_2$	14,887	10	6	20	10
$Man_7GlcNAc_2$	15,535	1		2	7

Sf9-1 to Sf9-3 and Tn5 are four preparations of hCD2ad variant **4**. % RA, percentage of total relative abundance. Sf9-1 to -3 represent different preparations of hCD2ad from Sf9 host. Tn5 is a different insect expression host; see *SI Appendix*.

Table S3. MS characterization of remodeled hCD2ad variants (see Fig. 1*C* and Fig. S1)

hCD2ad variant	$MW_{expected}$	MW_{found}	%	Structure	Enzyme step
5, sample 1	14,344	14,342	72	FucGlcNAc	Endo D cut
		14,195	28	GlcNAc	
5, sample 2	14,344	14,342	75	FucGlcNAc	Endo D/H cut
		14,195	25	GlcNAc	
6	14,708	14,709	44	$Man_1GlcNAc_2(Fuc)$	α-Mannosidase
		14,870	25	$Man_2GlcNAc_2(Fuc)$	
		14,723	17	$Man_2GlcNAc_2$	
		14,559	14	$Man_1GlcNAc_2$	
7	15,373	15,371	90	$Man_6GlcNAc_2$	Endo D uncut
		15,533	10	$Man_7GlcNAc_2$	
G	14,197	14,196		GlcNAc	Endo H cut
Paucimannose	15,034	15,031	100	$Man_3GlcNAc_2(Fuc)$	Endo H uncut
PNGase A or F treated	13,994	13,994	100	Asn65Asp	PNGase

Other Supporting Information Files

Dataset S1
SI Appendix