Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

1. Detailed experimental procedures.

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4. Comparison of OPA reagent to Nessler's reagent.

1. Detailed experimental procedures.

1.1 Preparation of OPA reagent. *o*-Phthalaldehyde (200 mg mL^{-1}) was dissolved in MeOH before dilution (1:100) into sodium tetraborate buffer (15 mM, pH 9.5). The reagent was stored at 5 ºC in the dark until use.

1.2 Microplate preparation. To a solution of nitrile substrate (100 mM in EtOH or DMSO, 23 µL) was added CFE solution (0.56 mg mL^{-1} in potassium phosphate buffer [10 mM, pH 7.2], 207 µL). Plates were covered and incubated overnight (18 h, 37 ºC).

1.3 Detection of ammonia. To a mixture of DMSO (140 µL) and OPA reagent (100 µL) was added the test solution (50 µL) and TCA solution (aq. 10% $^{w}/_{v}$, 50 µL). The resulting mixture was diluted into DMSO (1:2) and incubated (RT, 10 min). Absorbance of the solution at 675 nm measured using a BioTek Synergy HT multi-mode microplate reader. Concentration of ammonia was determined by comparison to a standard curve prepared using standard aqueous solutions of NH_4Cl (2-12 mM) and deionised water as a blank.

Fig. S1. Photograph of wells containing diluted test samples after acidification with (from left to right) acetic acid, hydrochloric acid, sulfuric acid, phosphoric acid, trichloroacetic acid and nitric acid (acids prepared as aq. 10% $^{w}/_{v}$ solutions and used as described in section 1.3).

1.4 Cloning of nitrilase coding sequences. Genes were amplified from genomic DNA using KOD Hot Start DNA polymerase (Merck Millipore) according to the manufacturer's user protocol. The following primer pairs were used to amplify the gene encoding the indicated enzyme (restriction sites for cloning are underlined) : 5' TG CCA TAG CAT ATG CAG ACA AGA AAA ATC GTC CG 3', 5' TG CCA TAG CTC GAG TCA GGA CGG TTC TTG CAC CA 3' (P20960); 5' TG CCA TAG GAA TTC ATG GAG GTT TTT AAT ATG ACA AGT ATT TAC CC 3', TG CCA TAG CTC GAG TTA CAC TTT TTC TTC AAG CAT ACC A 3' (B4AL96); 5' TG CCA TAG CAT ATG CAG GAC ACG AAA TTC AAA G 3', 5' TG CCA TAG CTC GAG TCA AGT CTC GGT GAA AGT GAC C 3' (Q89GE3); 5' TG CCA TAG CAT ATG GGA CTG GCA CAT CCG AA, 5' TG CCA TAG CTC GAG TCA GAT GGA TTG ATC GGG C 3'(A5EKU8); 5' TG CCA TAG CAT ATG TCC GCC AAA CTT ACC AA 3', 5' TG CCA TAG CTC GAG TTA CAC CTT TGC CTC AGC ATC T 3' (Q2GR86); 5' TG CCA TAG CAT ATG GCT AAG TTG AAA GTC GCG GCA GTT 3', 5' TG CCA TAG CTC GAG TCA GGC TCC GGC GTC GCC T 3' (Q6N284); 5' TG CCA TAG CAT ATG AAG ACG GAT TTG CGC GT 3', 5' TG CCA TAG CTC GAG TTA CTG GTC CTT GGC CTT GG 3' (A0LKP2); 5' TG CCA TAG GAA TTC ATG AGC AAT GAC CGC AGC TTT, 5' TG CCA TAG GTC GAC TCA TGC CGA GCC CCG CAC 3' (A5ETE9); 5' TG CCA TAG GGA TCC ATG ACG GAA ACC GCG CCC TT 3', 5' TG CCA TAG GAG CTC TCA TGT CTG CTC CCG CAC GA 3' (Q2J474); TG CCA TAG

CAT ATG CTT CCA TTG CAG GAT TTC CCA AAG 3', 5' TG CCA TAG CTC GAG CTA CTC GCT GGC CGG TTC 3' (Q5LLB2); 5' TG CCA TAG CAT ATG TCC ATG CAA CAG AGC TTC A 3', 5' TG CCA TAG CTC GAG TCA AGC CTC CTG TTT CTG GC 3' (E3HN55); 5' TG CCA TAG CAT ATG CTC GAA TTG CCG AAA 3', 5' TG CCA TAG CTC GAG TCA TTG GGA TGC CTC CTG 3' (F0Q9Y1); 5' TG CCA TAG CAT ATG CAG ACT CGT AAA ATT GTT CGT G 3', 5' TG CCA TAG CTC GAG GGA CGG TTC CTG AAC CAG CAG A 3' (G8CXY5); 5' TG CCA TAG CAT ATG AAA GTC GCA TGT ATT C 3', 5' TG CCA TAG CTC GAG TTA TTT GTA TAA TTC TGG AC 3' (Q819F0); 5' TG CCA TAG CAT ATG TTA AAT TAT TAT AGG AGT GAT ATT ATG GC 3', 5' TG CCA TAG CTC GAG TTA TTC ATG TAC AAT AAG TTC AAA TAC ATC AG 3' (A5MYU1); 5' TG CCA TAG CAT ATG ATG GCA AAG AAG GTT GTA GC 3', 5' TG CCA TAG CTC GAG CTA CTT AAT AAA AAC AGT ATC GTG ACT C 3' (C5DH06); 5' TG CCA TAG CAT ATG TGC TGG TGT TAT CCT GGT TGC AAA T 3', 5' TG CCA TAG CTC GAG CTA CCG AAC CGG TAC AGC CTG CTT 3' (D1C8L7); 5' TG CCA TAG CAT ATG TCT TCT ATA TTA TCT CAA AAG TTA AAA GTT G 3', 5' TG CCA TAG CTC GAG TCA TTT GTT AAC ATC AGG ATA GAT ATC G 3' (A7TP07). All PCR products were cloned into pET-28a using the restriction sites engineered into the primers.

1.5 Cell free extract production. Recombinant proteins were expressed from *E. coli* BL21(DE3). Cells were grown at 37 ºC with shaking at 200 rpm in LB medium supplemented with 100 mg mL^{-1} kanamycin to an absorbance of 0.6 at 600 nm. Induction was performed by the addition of isopropyl-1-thio-*β*-D-galactopyranoside, to a concentration of 240 mg mL^{-1}, followed by further incubation for 18 h at 20 ºC, 100 rpm. Cells were harvested by centrifugation (15 min, 4000 x g, 4 ºC), resuspended in $^{1}/_{10}$ volume of 18.2 MΩ cm^{-1} water and lysed by sonication (6 x 10 s at 14 microns). The resulting cell free extracts were cleared by centrifugation (20 min, 20000 x g, 4 ºC) and stabilised by freeze-drying.

1.6 Example of an ongoing assay.

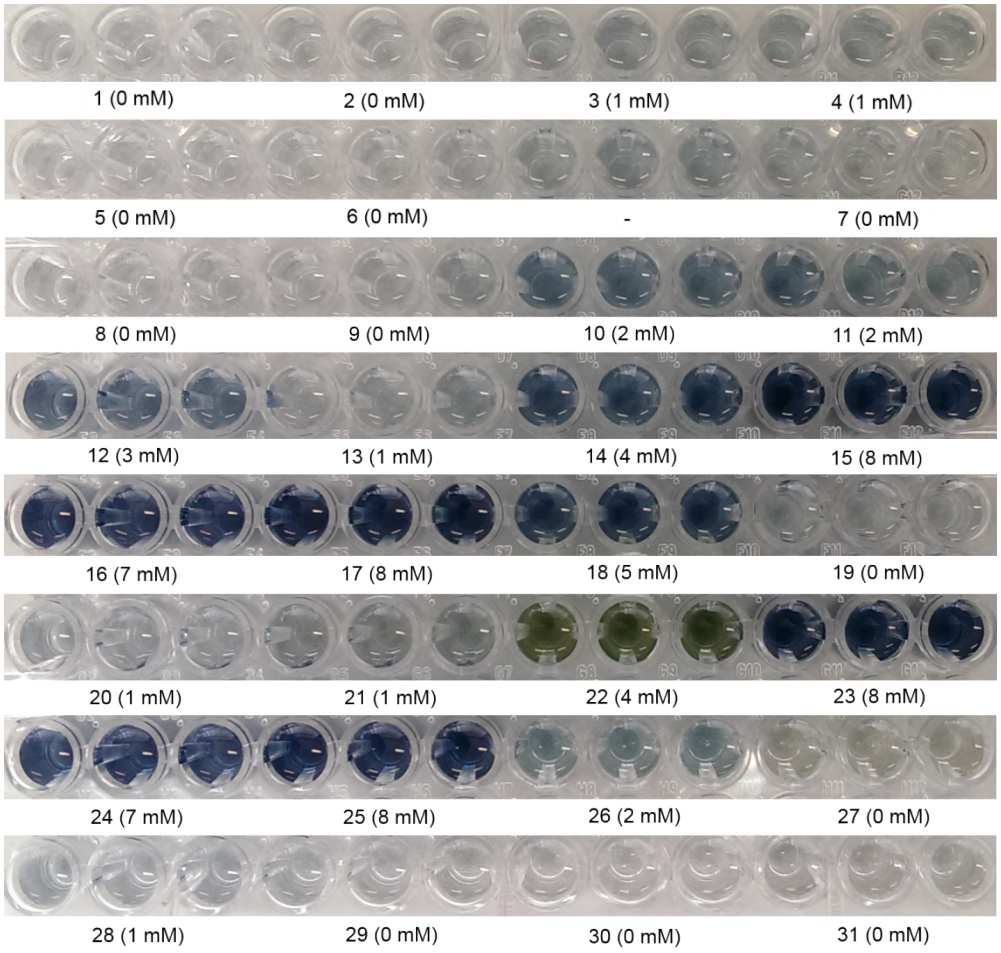

Fig. S2. An example of the output of this assay. Wells shown are charged in triplicate with the reaction mixture from nitrilase 5 and thirty-one of the substrates used in this screen (including one commercially-sensitive example). Substrate 22 (4-aminobenzonitrile) is chromogenic itself with OPA reagent, however the yellow colour produced does not interfere with quantitation of the OPA chromophore. (This figure shows exactly the same microplate as Figure 3 but has been sliced to include compound number and ammonia concentration data)

2. Rational sampling of sequence space.

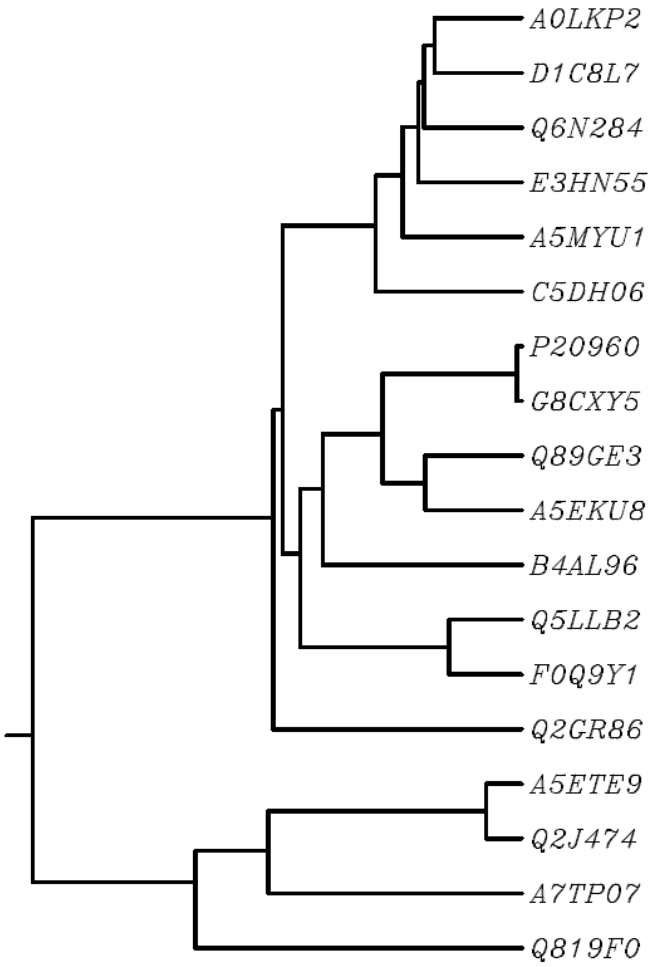

Fig. S3. A rooted phylogenetic tree of the rationally sampled 18 nitrilases included in this screen. The primary amino acid sequence identity varies between 6-96%, with a mean identity of 20%.

2. Full Tables of Screening Results

Table S1a. Screening of nitrilases (Number and depth of shading correspond to ammonia concentration in mM. Maximum 10 mM.).

No.	Entry	1	2	3a	3b	4	5	6a	6b	7	8	9	10a	10b
	Organism (Uniprot code provided in brackets)	*Alcaligenes faecalis* (P20960)	*Bacillus pumilus* (B4AL96)	*Bradyrhizobium japonicum* (Q89GE3)	*Bradyrhizobium japonicum* (Q89GE3)	*Bradyrhizobium* sp. BTAi1 (A5EKU8)	*Chaetomium globosum* (Q2GR86)	*Rhodopseudomonas palustris* (Q6N284)	*Rhodopseudomonas palustris* (Q6N284)	*Syntrophobacter fumaroxidans* (A0LKP2)	*Bradyrhizobium* sp. BTAi1 (A5ETE9)	*Rhodopseudomonas palustris* (Q2J474)	*Silicibacter pomeroyi* (Q5LLB2)	*Silicibacter pomeroyi* (Q5LLB2)
	aliphatic substrates													
1	acetonitrile	0	0	0	0	1	0	0	0	0	0	0	0	0
2	propionitrile	0	0	0	0	0	0	0	0	0	0	0	0	0
3	butyronitrile	0	0	0	0	0	1	0	0	0	0	0	0	0
4	valeronitrile	0	0	0	0	2	1	0	0	0	0	0	0	0
5	hexanenitrile	0	0	2	1	3	0	0	0	0	0	0	0	0
6	isobutyronitrile	0	0	0	0	0	0	0	0	0	0	0	0	0
7	methacrylonitrile	0	0	0	0	1	0	0	0	0	0	0	0	0
8	acrylonitrile	0	0	0	0	1	0	0	0	0	0	0	0	0
9	cyclohexanecarbonitrile	0	0	0	0	0	0	0	0	0	0	0	1	0
	aromatic substrates													
10	benzonitrile	0	0	0	0	0	2	0	0	0	1	0	1	0
11	2-hydroxybenzonitrile	0	0	0	0	0	2	0	0	0	0	0	0	0
12	4-hydroxybenzonitrile	0	0	0	0	0	3	0	0	0	0	0	0	0
13	*o*-tolunitrile	0	0	0	0	0	1	0	0	0	0	0	0	0
14	*p*-tolunitrile	0	0	0	0	0	4	0	0	0	0	0	0	0
15	4-methoxybenzonitrile	0	0	0	0	0	8	0	0	0	0	0	0	0
16	4-chlorobenzonitrile	0	0	0	0	0	7	0	0	0	0	0	0	0
17	4-dimethylaminobenzonitrile	0	0	0	0	0	8	0	0	0	0	0	0	0
18	4-trifluoromethylbenzonitrile	0	0	0	0	0	5	0	0	0	0	0	0	0
19	2,6-dichlorobenzonitrile	0	0	0	0	0	0	0	0	0	0	0	0	0
20	3,5-diisopropyl-4-dimethylaminobenzonitrile	0	0	0	0	0	1	0	0	0	0	0	0	0
21	2-aminobenzonitrile	0	0	1	1	1	1	0	0	0	0	0	0	0
22	4-aminobenzonitrile	0	0	1	1	1	4	0	0	0	0	0	0	0
23	picolinonitrile	0	0	2	1	4	8	1	1	0	0	1	0	0
24	nicotinonitrile	0	0	1	1	2	7	0	0	0	0	0	0	0
25	isonicotinonitrile	0	0	3	1	3	8	1	1	1	0	0	0	0
26	isophthalonitrile	2	0	0	0	1	3	0	1	0	0	0	0	0
	arylaliphatic substrates													
27	phenylacetonitrile	0	0	10	8	10	2	1	2	0	0	0	0	1
28	4-nitrophenylacetonitrile	0	0	8	6	8	0	2	6	4	0	0	0	0
29	2-chlorophenylacetonitrile	0	0	9	6	7	1	0	0	0	0	0	0	0
30	2-(2-nitrophenyl)acetonitrile	0	0	8	6	6	0	0	0	0	0	0	0	0
31	2-(2,6-dichlorophenyl) acetonitrile	0	0	8	6	3	0	0	0	0	0	0	0	0
32	benzoylacetonitrile	0	1	4	4	4	0	1	1	0	0	0	0	0
33	2-phenylpropionitrile	1	0	7	5	4	0	0	0	0	0	0	0	0
34	mandelonitrile	0	5	6	5	4	0	0	0	0	0	0	1	0
35	ibuprofen nitrile	0	0	1	1	1	0	0	1	0	0	0	1	0
36	2-diphenylacetonitrile	0	0	1	1	1	0	0	0	0	0	0	0	0
37	2-(2-naphthyl)acetonitrile	0	1	5	4	0	0	0	0	0	0	0	0	0
38	3-phenylpropionitrile	1	0	7	5	8	2	1	4	1	0	0	1	0

Table S1b. Screening of nitrilases (Number and depth of shading correspond to ammonia concentration in mM. Maximum 10 mM.).

	Organism (Uniprot code provided in brackets)	*Achromobacter xylosoxidans* (E3HN55)	*Acidovorax avenae* (F0Q9Y1)	*Alcaligenes faecalis* (G8CXY5)	*Bacillus cereus* (Q819F0)	*Clostridium kluyveri* (A5MYU1)	*Lachancea thermotolerans* (C5DH06)	*Sphaerobacter thermophilus* (D1C8L7)	*Vanderwaltozyma polyspora* (A7TP07)
No.	**Entry**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
	aliphatic substrates								
1	acetonitrile	0	0	0	0	0	0	0	0
2	propionitrile	0	0	0	0	0	0	0	0
3	butyronitrile	0	0	0	0	0	0	0	0
4	valeronitrile	0	0	0	0	0	0	0	0
5	hexanenitrile	0	0	0	0	0	0	0	0
6	isobutyronitrile	0	0	0	0	0	0	0	0
7	methacrylonitrile	0	0	0	0	0	0	0	0
8	acrylonitrile	0	0	0	0	0	0	0	0
9	cyclohexanecarbonitrile	0	0	0	0	0	0	0	0
	aromatic substrates								
10	benzonitrile	0	0	0	0	0	0	0	0
11	2-hydroxybenzonitrile	0	0	0	0	0	0	0	0
12	4-hydroxybenzonitrile	0	0	0	0	0	0	0	0
13	*o*-tolunitrile	0	0	0	0	0	0	0	0
14	*p*-tolunitrile	0	0	0	0	0	0	0	0
15	4-methoxybenzonitrile	0	0	0	0	0	0	0	0
16	4-chlorobenzonitrile	0	0	0	0	0	0	0	0
17	4-dimethylaminobenzonitrile	0	0	0	0	0	0	0	0
18	4-trifluoromethylbenzonitrile	0	0	0	0	0	0	0	0
19	2,6-dichlorobenzonitrile	0	0	0	0	0	0	0	0
20	3,5-diisopropyl-4-dimethylaminobenzonitrile	0	0	0	0	0	0	0	0
21	2-aminobenzonitrile	0	0	0	0	0	0	0	0
22	4-aminobenzonitrile	0	0	0	0	0	0	0	0
23	picolinonitrile	0	0	0	0	0	0	0	0
24	nicotinonitrile	0	0	0	0	0	0	0	0
25	isonicotinonitrile	0	0	0	0	0	0	0	0
26	isophthalonitrile	0	0	0	0	0	0	0	0
	arylaliphatic substrates								
27	phenylacetonitrile	0	0	0	0	0	0	0	0
28	4-nitrophenylacetonitrile	0	0	0	0	0	0	0	0
29	2-chlorophenylacetonitrile	0	0	0	0	0	0	0	0
30	2-(2-nitrophenyl)acetonitrile	0	0	0	0	0	0	0	0
31	2-(2,6-dichlorophenyl) acetonitrile	0	0	0	0	0	0	0	0
32	benzoylacetonitrile	0	0	0	0	0	0	0	0
33	2-phenylpropionitrile	0	0	0	0	0	0	0	0
34	mandelonitrile	0	0	0	0	0	0	0	0
35	ibuprofen nitrile	0	0	0	0	0	0	0	0
36	2-diphenylacetonitrile	0	0	0	0	0	0	0	0
37	2-(2-naphthyl)acetonitrile	0	0	0	0	0	0	0	0
38	3-phenylpropionitrile	0	0	0	0	0	0	0	0

Table S2. Screening of Prozomix nitrilases (Number and depth of shading correspond to ammonia concentration in mM. Maximum 10 mM.).

	Organism (Uniprot code provided in brackets)	*PRO-NITR010*	*PRO-NITR011*	*PRO-NITR012*	*PRO-NITR014*	*PRO-NITR018*
No.	**Entry**	**19**	**20**	**21**	**22**	**23**
	aliphatic substrates					
1	acetonitrile	1	0	1	0	0
2	propionitrile	0	0	0	0	0
3	butyronitrile	1	0	0	0	0
4	valeronitrile	1	0	0	0	0
5	hexanenitrile	2	0	0	0	0
6	isobutyronitrile	1	0	0	0	0
7	methacrylonitrile	1	0	0	0	0
8	acrylonitrile	1	0	0	0	0
9	cyclohexanecarbonitrile	1	0	0	0	1
	aromatic substrates					
10	benzonitrile	2	0	0	1	0
11	2-hydroxybenzonitrile	3	0	0	0	0
12	4-hydroxybenzonitrile	2	0	0	0	0
13	*o*-tolunitrile	1	0	0	0	0
14	*p*-tolunitrile	5	0	0	0	0
15	4-methoxybenzonitrile	8	0	0	0	0
16	4-chlorobenzonitrile	9	0	0	1	0
17	4-dimethylaminobenzonitrile	8	0	0	0	0
18	4-trifluoromethylbenzonitrile	7	0	0	2	0
19	2,6-dichlorobenzonitrile	1	0	0	0	0
20	3,5-diisopropyl-4-dimethylaminobenzonitrile	1	0	0	1	0
21	2-aminobenzonitrile	2	0	0	1	0
22	4-aminobenzonitrile	2	0	0	1	0
23	picolinonitrile	9	1	0	6	0
24	nicotinonitrile	5	1	0	9	0
25	isonicotinonitrile	8	1	0	9	0
26	isophthalonitrile	2	0	0	2	0
	arylaliphatic substrates					
27	phenylacetonitrile	2	1	0	1	0
28	4-nitrophenylacetonitrile	1	1	0	1	0
29	2-chlorophenylacetonitrile	1	1	0	1	0
30	2-(2-nitrophenyl)acetonitrile	1	0	0	1	0
31	2-(2,6-dichlorophenyl) acetonitrile	0	1	0	0	0
32	benzoylacetonitrile	1	2	1	1	0
33	2-phenylpropionitrile	0	1	0	0	0
34	mandelonitrile	0	1	0	0	0
35	ibuprofen nitrile	0	0	0	0	0
36	2-diphenylacetonitrile	0	0	0	0	0
37	2-(2-naphthyl)acetonitrile	0	1	0	0	0
38	3-phenylpropionitrile	7	1	0	0	1

3. Comparison of OPA reagent to Nessler's reagent.

3.1 Reagent and microplate preparation: OPA reagent and microplates were prepared as outlined in section **1.1-1.2** using substrates listed in **table S4** and lyophilised CFE of entries **3a**, **5**, **19** and **22** (**table S1**). Ammonia concentration was determined using Nessler's reagent (section **3.2**) and also using OPA reagent as outlined in section **1.3**.

3.2 Determination of ammonia concentration by Nessler's reagent: Test solutions (10 µL) were diluted into deionised water (170 µL) and Nessler's reagent (20 µL) added. After incubation (RT, 15 min) absorbance at 425 nm was determined using a BioTek Synergy HT multi-mode microplate reader. Concentration of ammonia was determined by comparison to a standard curve prepared using standard aqueous solutions of NH_4Cl (2-12 mM) and deionised water as a blank. Some solutions developed turbidity on incubation and no accurate absorbance was obtained.

3.3 Results of comparison of OPA reagent and Nessler's reagent for the determination of ammonia concentration.

Table S3. Absorbance of standard NH_4Cl solutions with both reagents.

NH_4Cl conc. / mM	A_{675} using OPA reagent	A_{425} using Nessler's reagent
0	0.000	0.000
2	0.157	0.149
4	0.374	0.303
6	0.635	0.492
8	0.879	0.643
10	1.045	0.850
12	1.375	1.021

Chart S1. Calibration curve for OPA reagent.

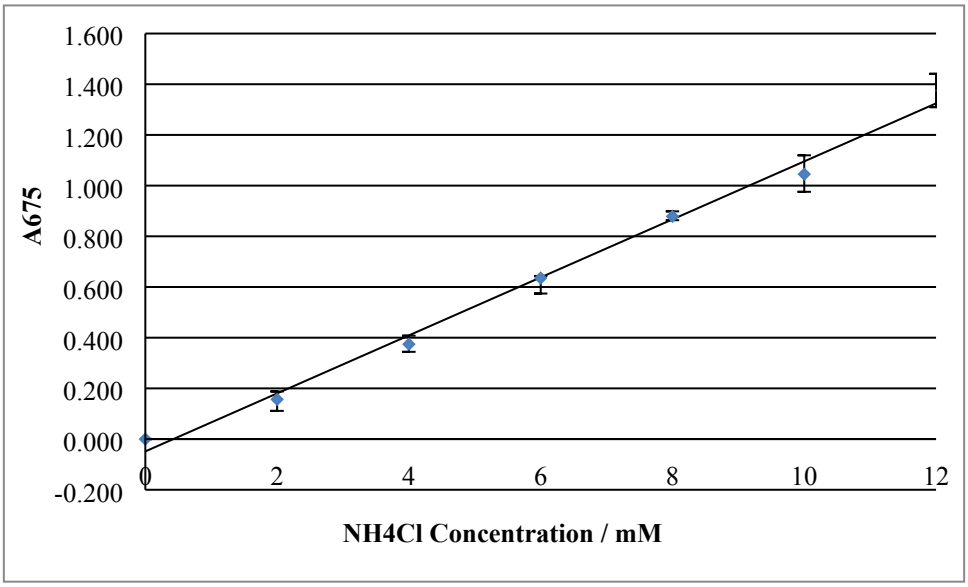

Chart S2. Calibration curve for Nessler's reagent.

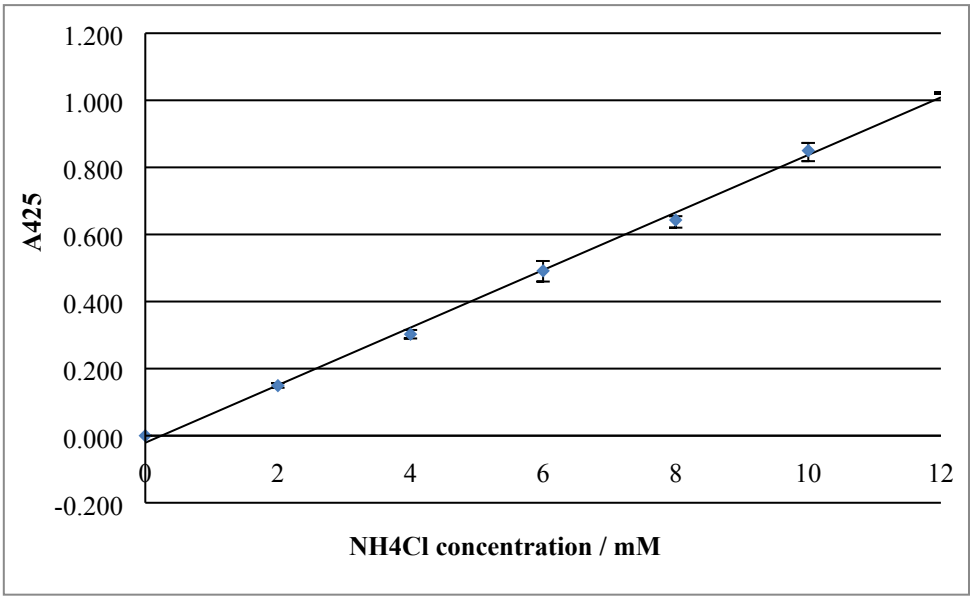

Table S4. Ammonia concentration determined by both OPA and Nessler's reagent.

Substrate	NH_3 concentration by Nessler's reagent / mM				NH_3 concentration by OPA reagent / mM			
	3a	**5**	**19**	**22**	**3a**	**5**	**19**	**22**
acetonitrile	0	1	2	0	0	1	2	1
propionitrile	0	1	1	0	0	1	1	1
butyronitrile	0	1	1	1	0	1	1	1
valeronitrile	1	1	1	0	1	1	1	1
hexanenitrile	1	1	2	0	1	1	2	1
isobutyronitrile	0	1	1	0	0	1	1	1
methacrylonitrile	0	1	1	0	0	1	1	1
acrylonitrile	0	1	1	0	0	1	1	1
cyclohexanecarbonitrile	0	2	2	0	0	2	2	1
benzonitrile	0	3	2	2	0	3	1	2
2-hydroxybenzonitrile	0	2	1	1	0	2	1	1
4-hydroxybenxonitrile	0	1	1	0	0	2	1	1
p-tolunitrile	0	4	4	1	0	4	4	1
4-methoxybenzonitrile	0	9	5	1	0	9	4	1
4-chlorobenzonitrile	0	7	8	1	0	6	6	1
4-(dimethylamino)benzonitrile	0	7	6	1	0	7	5	1
4-(trifluoromethyl)benzonitrile	0	5	5	2	0	5	5	2
3,5-diisopropyl-4-(dimethylamino) benzonitrile	turbid	turbid	turbid	1	0	2	1	1
2-aminobenzonitrile	1	turbid	turbid	1	1	2	1	1
picolinonitrile	1	10	turbid	turbid	1	10	6	3
nicotinonitrile	1	9	3	7	1	9	2	7
isonicotinonitrile	2	9	2	5	1	8	1	6
phenylacetonitrile	8	turbid	turbid	turbid	6	1	1	1
2-(chlorophenyl)acetonitrile	9	4	turbid	turbid	7	2	1	1
2-phenylpropionitrile	5	2	1	1	5	2	1	1
mandelonitrile	4	turbid	1	1	4	1	1	1
ibuprofen nitrile	1	1	1	0	1	0	0	0
3-phenylpropionitrile	8	4	8	1	9	3	7	1
isophthalonitrile	1	1	1	1	1	1	0	0